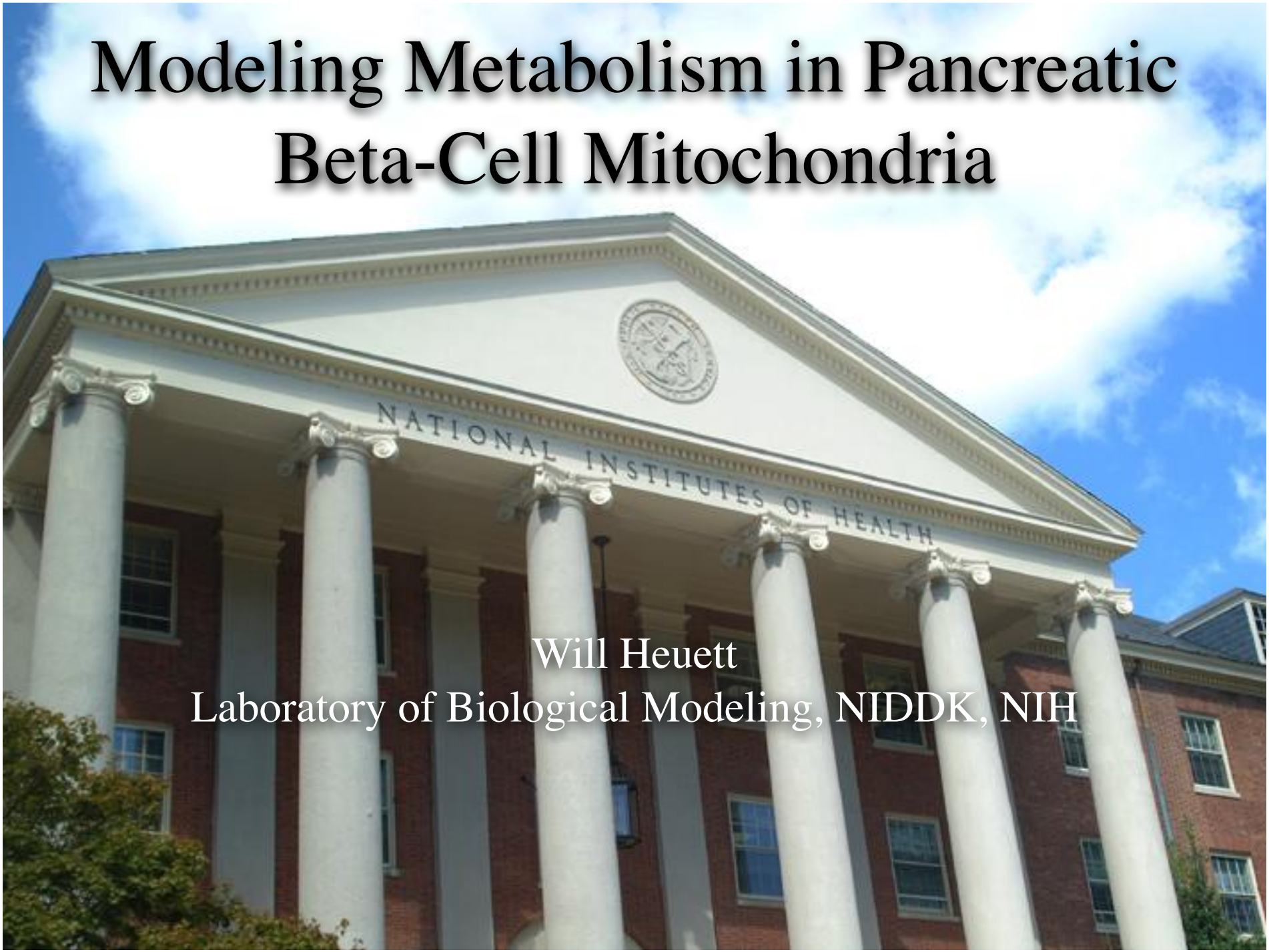
Modeling Metabolism in Pancreatic Beta-Cell Mitochondria



Will Heuett
Laboratory of Biological Modeling, NIDDK, NIH

METRICS

BILL MARSH

The New York Times

August 3, 2008

The Overflowing American Dinner Plate

IN 1970, the average American ate about 16.4 pounds of food a week, or 2.3 pounds daily. By 2006, the average intake grew by an additional 1.8 pounds a week.

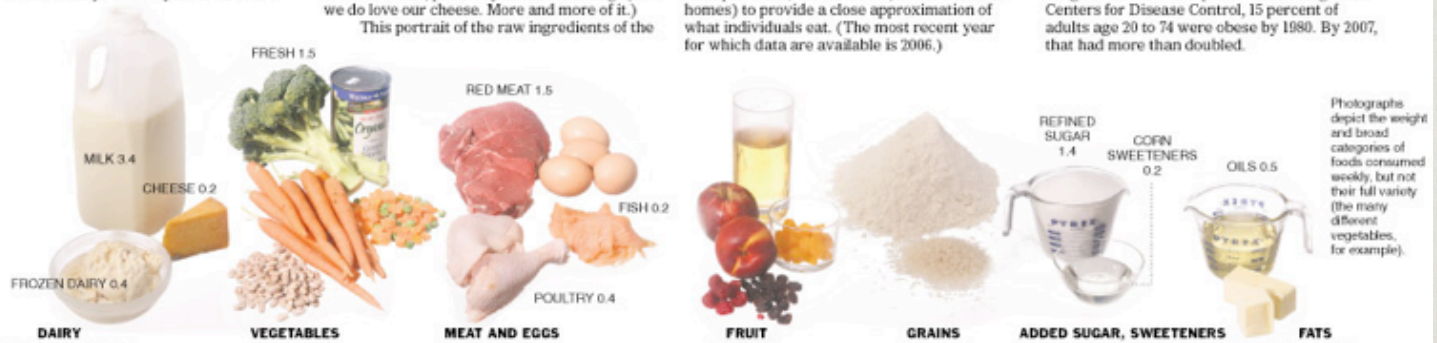
Among other things, that's an extra half pound of fat weekly — mostly from oils and shortening. That doesn't count the fat in the extra quarter pound of meat Americans now eat every seven days. Those fats were somewhat offset by a steep drop in dairy consumption, the only major food group to have a decline, primarily in milk drinking. (But we do love our cheese. More and more of it.)

This portrait of the raw ingredients of the American diet is based on what the Agriculture Department calls "food availability" — the amount of food produced for the average American consumer. The data are adjusted for food losses (waste on farms; in processing and transportation; and in stores, restaurants and homes) to provide a close approximation of what individuals eat. (The most recent year for which data are available is 2006.)

The numbers don't reveal how much grain went into bread versus cookies, or how many chicken breasts became chicken nuggets. But the overall increase in eating does suggest a link with the rise in Americans' weight over the same period. According to the Centers for Disease Control, 15 percent of adults age 20 to 74 were obese by 1980. By 2007, that had more than doubled.

The Weekly Diet in 1970...

FIGURES IN APPROX. POUNDS



...And How It Changed By 2006

Whole milk consumption plunged, with lower-fat milks replacing only some of that; soft drinks and bottled water are now preferred beverages.

Americans are eating more vegetables, but still not enough to meet federal recommendations.

Red meat is down; poultry is way up. Fish, growing fastest, is less than 10 percent of the category.

Nearly half of fruit consumption is in the form of juice, with some of that used as a food sweetener.

Almost 90 percent are refined grains; government guidelines call for far more whole grains.

This is what goes into processed foods (not sugars occuring naturally in fruit and milk).

The fastest-growing of these food categories, it includes both oils and some animal fats.

Percentage Change in Consumption of Selected Foods, 1970-2006

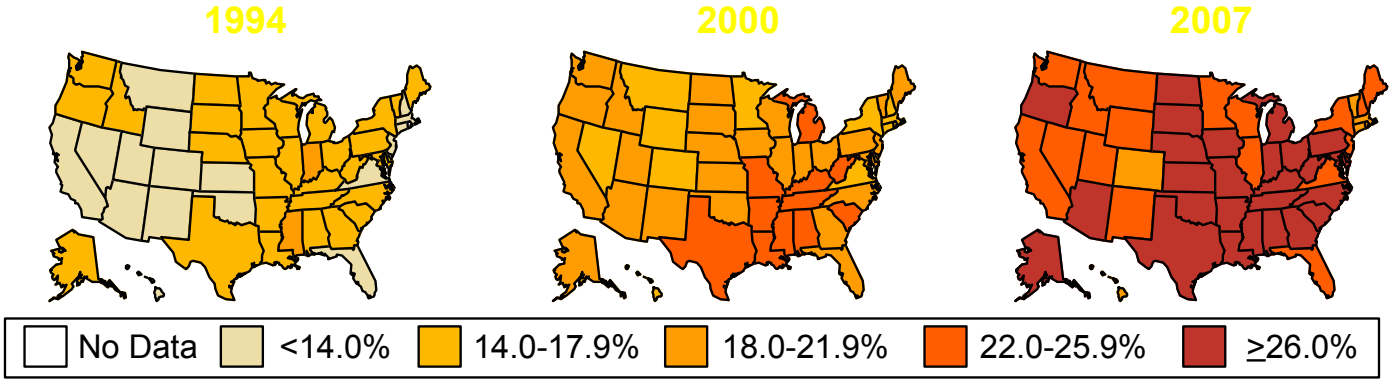
Other statistics on American food consumption are at www.ers.usda.gov/Data/FoodConsumption under "Loss-Adjusted Food Availability."

Source: United States Department of Agriculture

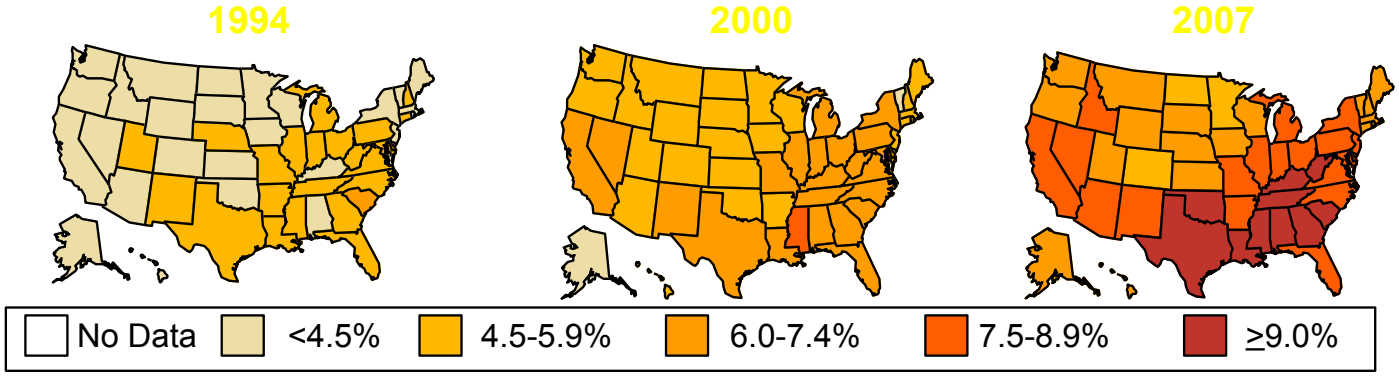
PHOTOGRAPHS BY TONY CENICOLA/THE NEW YORK TIMES

Age-adjusted Percentage of U.S. Adults Who Were Obese or Who Had Diagnosed Diabetes

Obesity (BMI ≥30 kg/m^2)



Diabetes

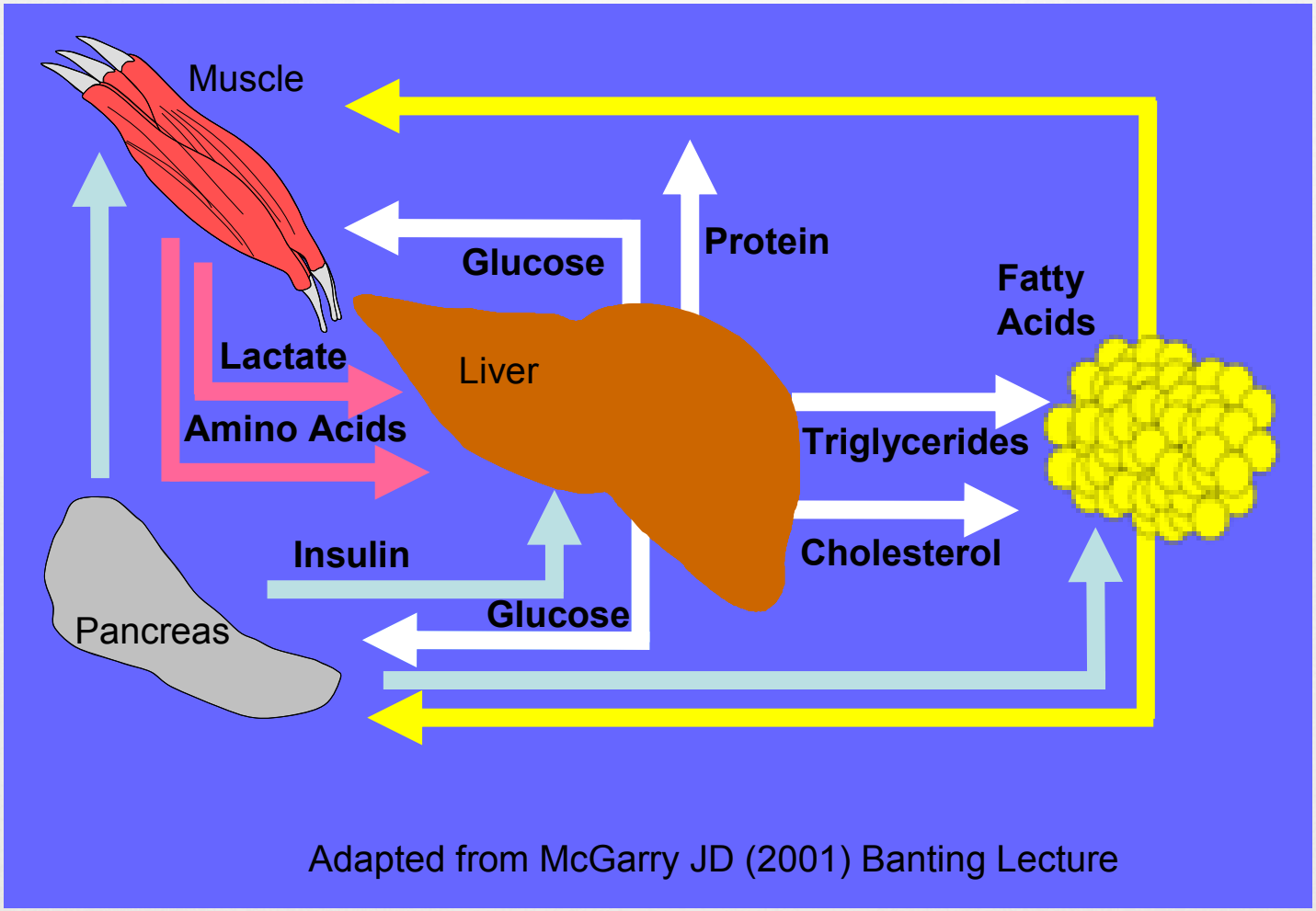


CDC's Division of Diabetes Translation. National Diabetes Surveillance System available at http://www.cdc.gov/diabetes/statistics

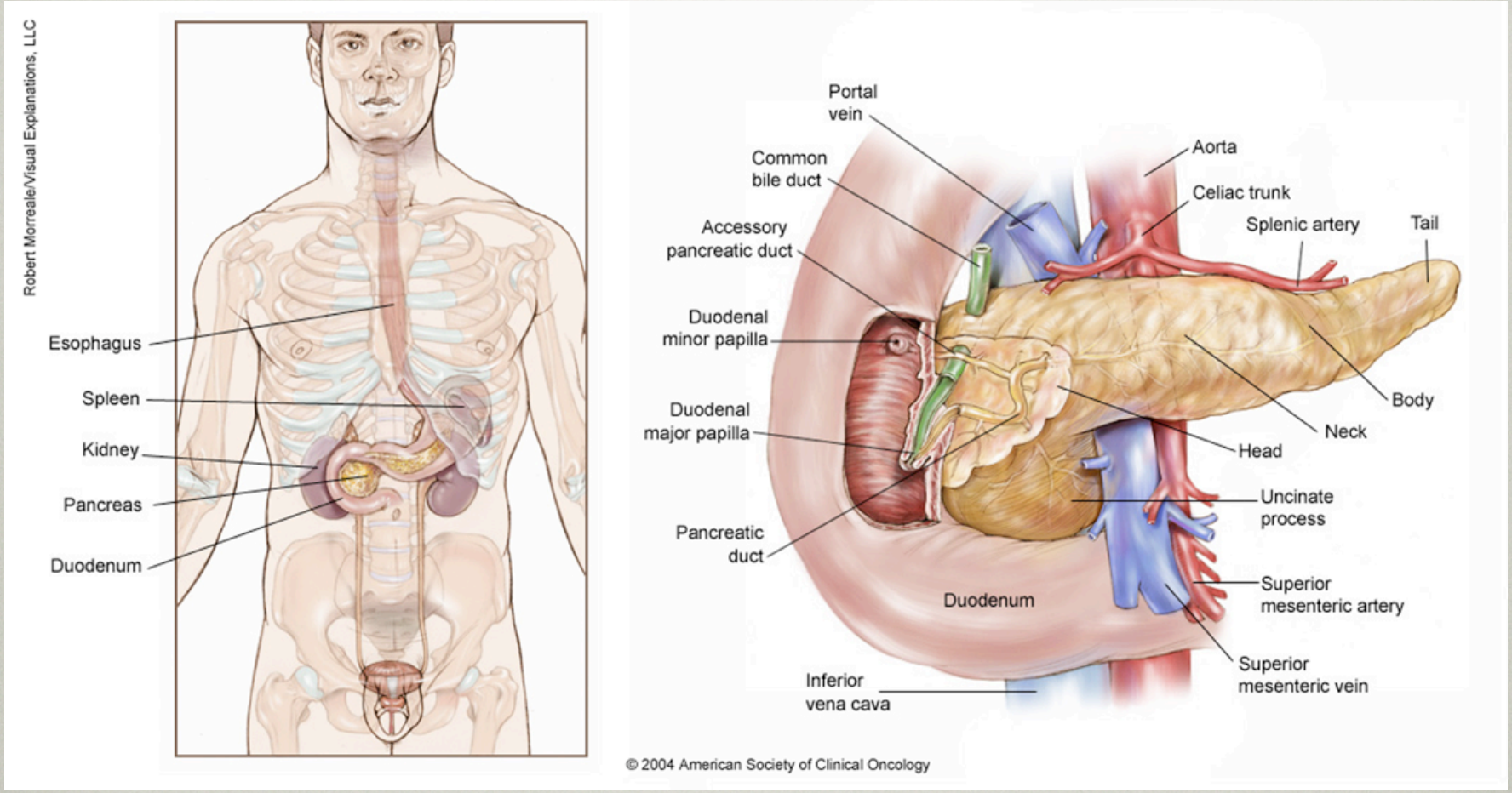
Obesity increases risk of:

- Coronary heart disease
- Type 2 diabetes
- Cancers (endometrial, breast, and colon)
- Hypertension (high blood pressure)
- Dyslipidemia (for example, high total cholesterol or high levels of triglycerides)
- Stroke
- Liver and Gallbladder disease
- Sleep apnea and respiratory problems
- Osteoarthritis (a degeneration of cartilage and its underlying bone within a joint)
- Gynecological problems (abnormal menses, infertility)

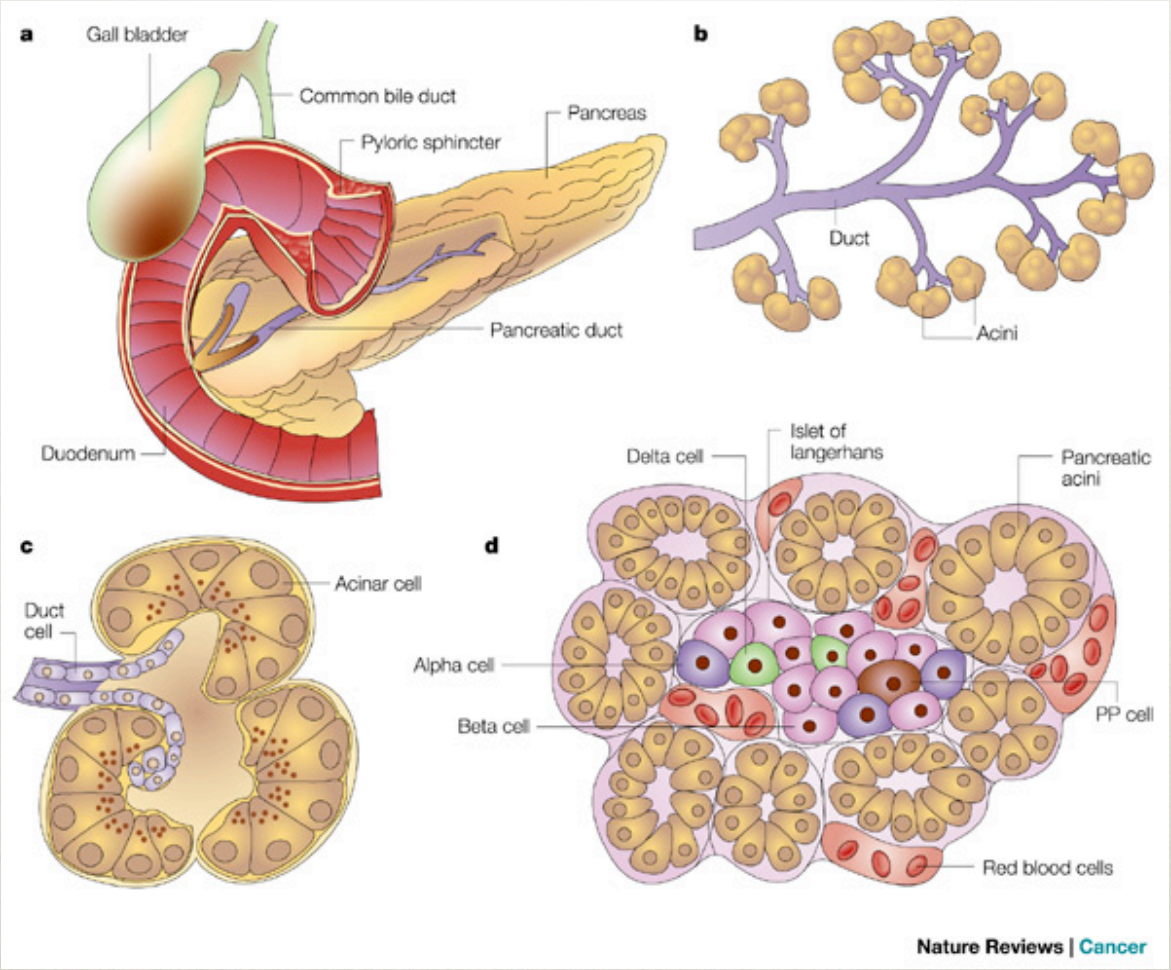
Whole Body Metabolism



The Pancreas

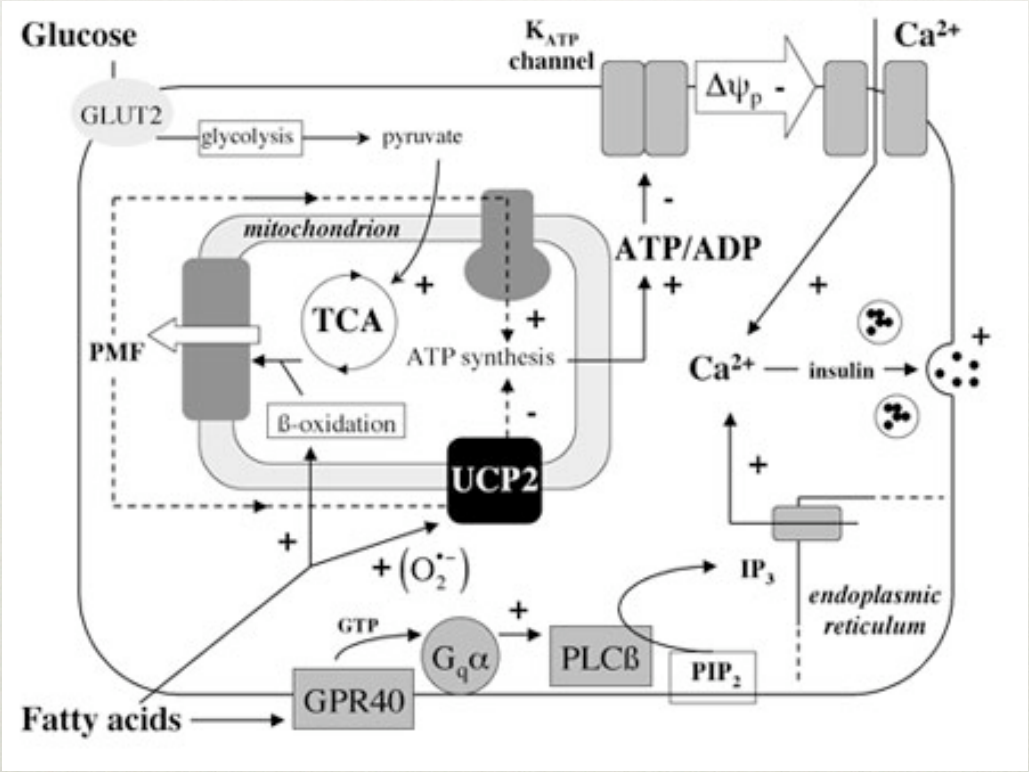


Islets of Langerhans



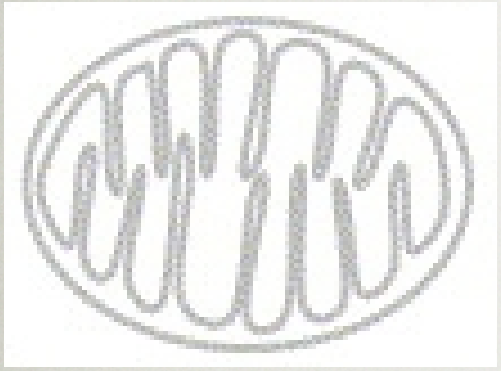
Bardeesy, N and RA DePinho. *Nature Reviews Cancer*, **2**:897-990, 2002.

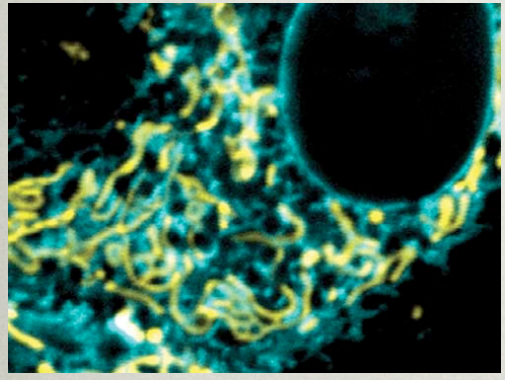
The Pancreatic β-cell



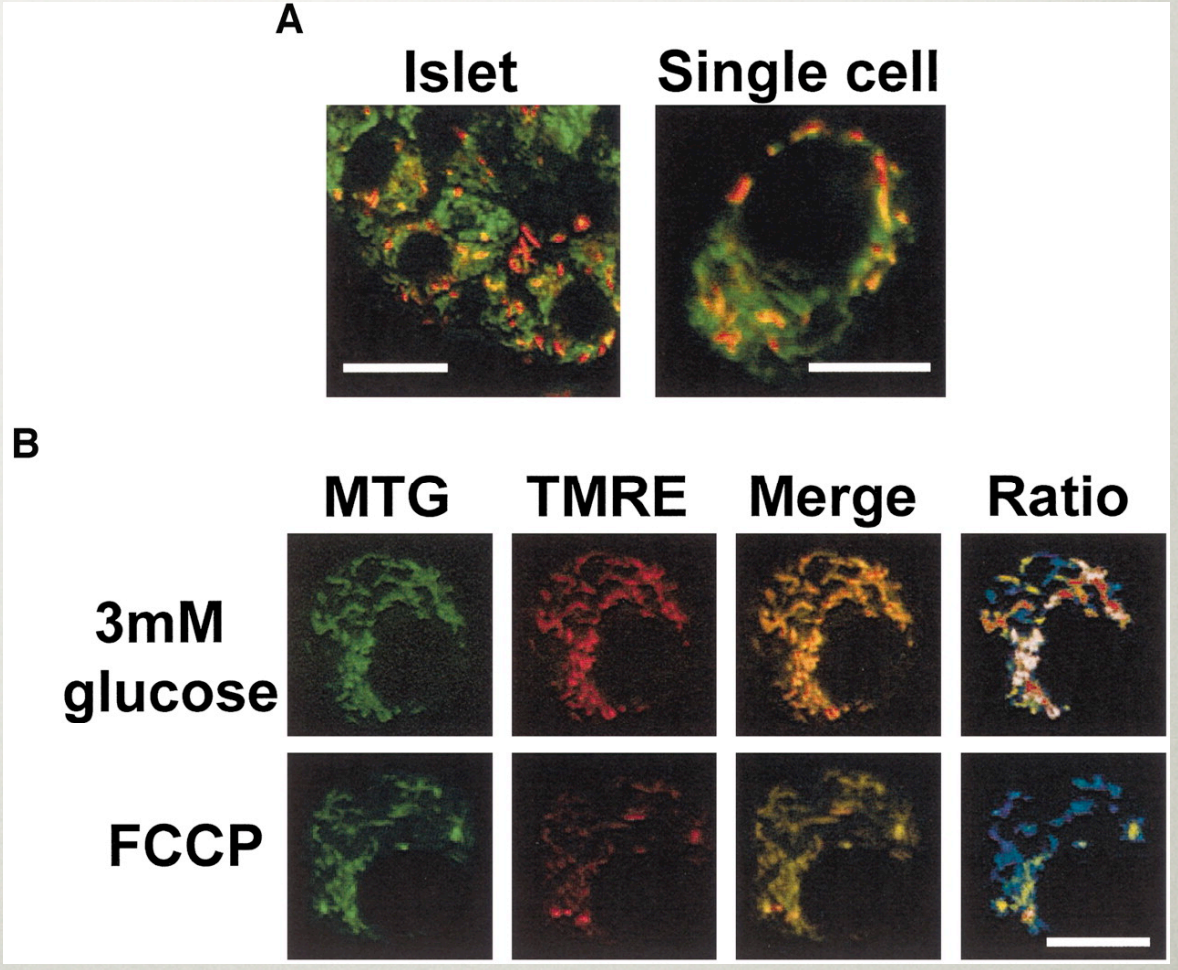
Brand, MD *et al*. *Free Radic*. *Biol*. *Med*., **37**:755-767, 2004.

Mitochondria





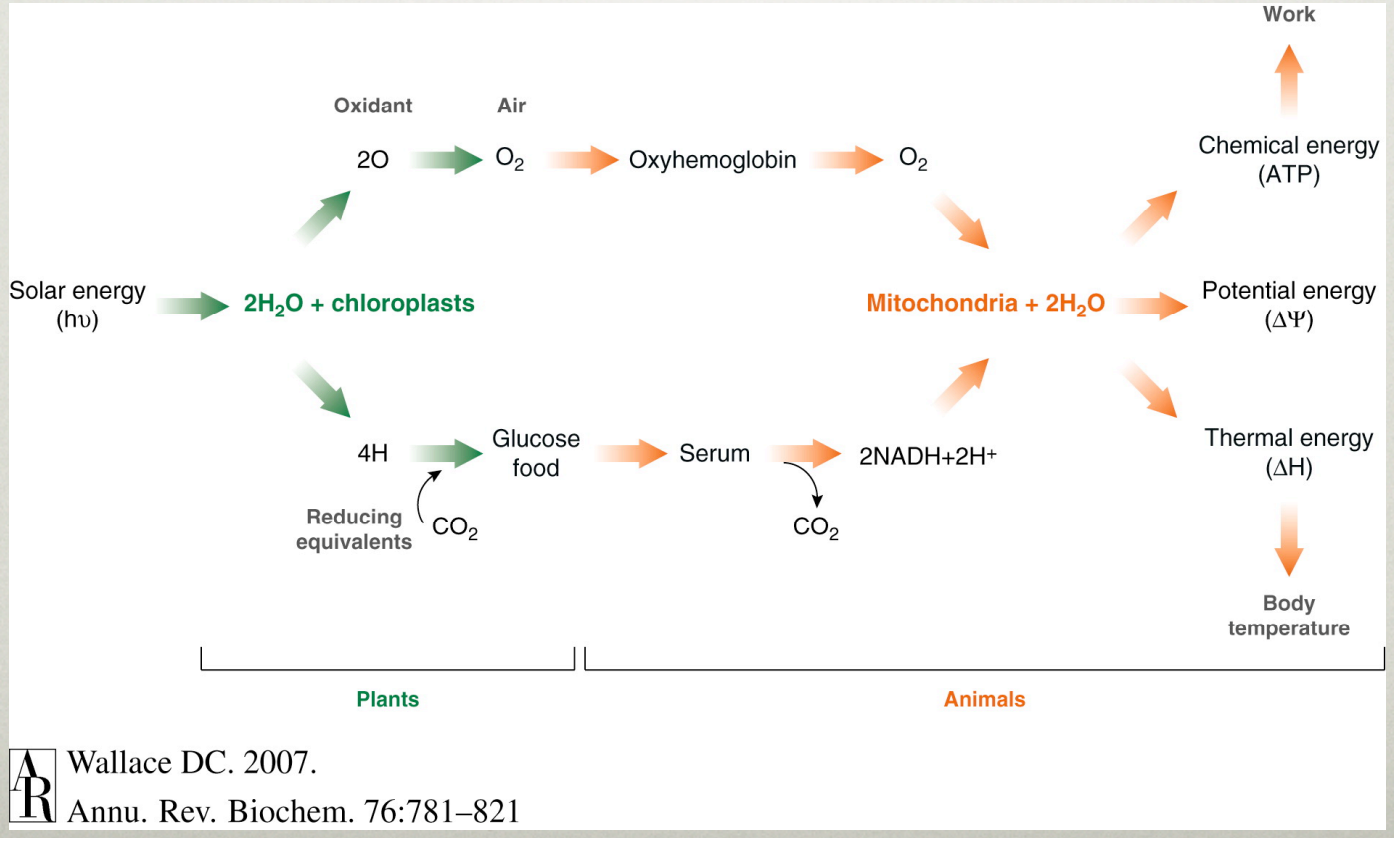
Piston, D, Vanderbilt University.



Wikstrom *et al.*, *Diabetes*, **56**:2569-2578, 2007.

History of Mitochondria

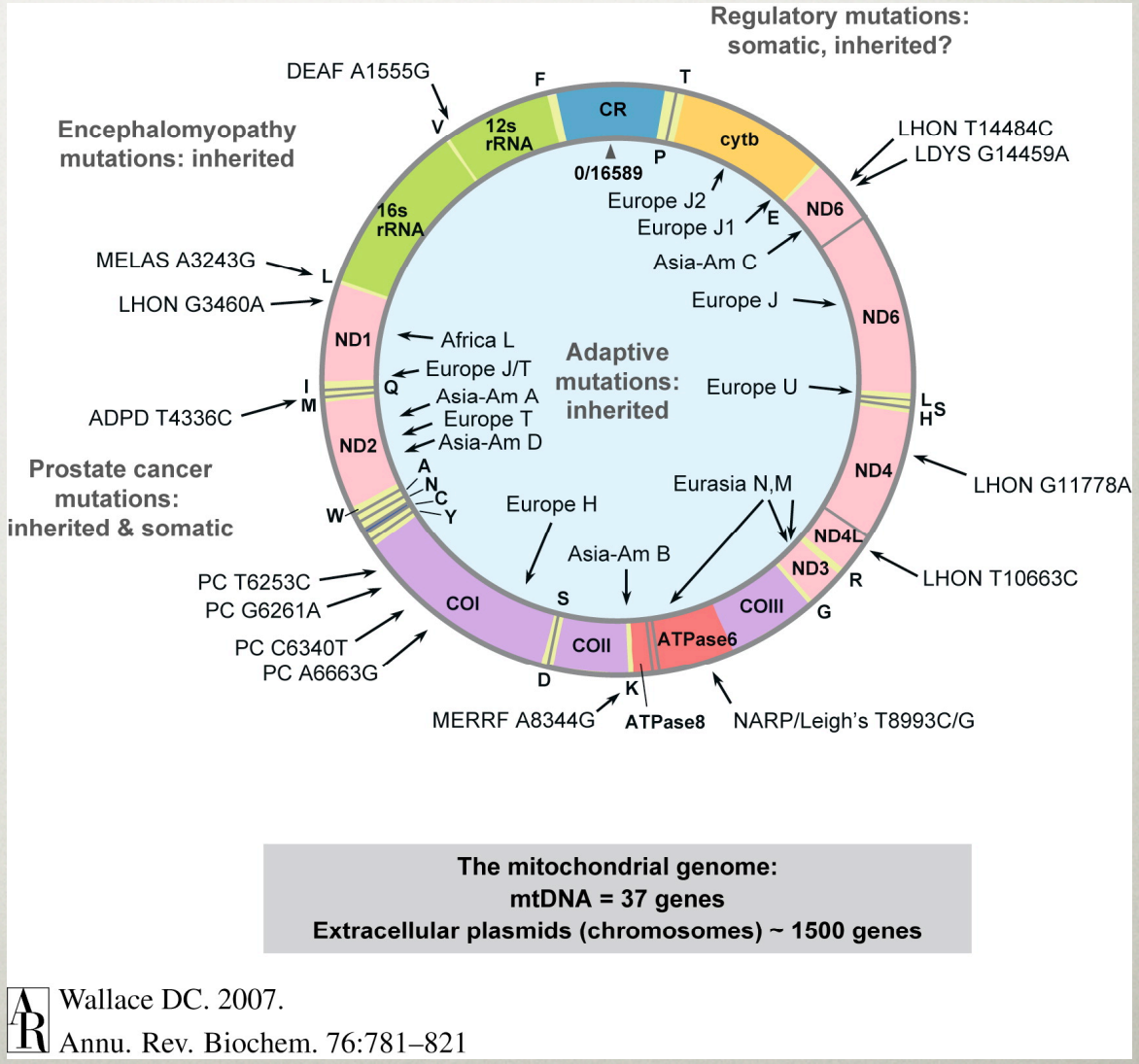
- Eukaryotic ancestors engulfed or were infected by ancient bacteria ~ 2 billion years ago in symbiosis.
- Structure, energy, and information.



Wallace DC. 2007.
Annu. Rev. Biochem. 76:781–821

Mitochondrial DNA

- 37 genes = 13 subunits of OXPHOS + 22 tRNA + 2 rRNA.
- ~ 2–10 mtDNA copies per mitochondria and 100's of mitochondria per cell.



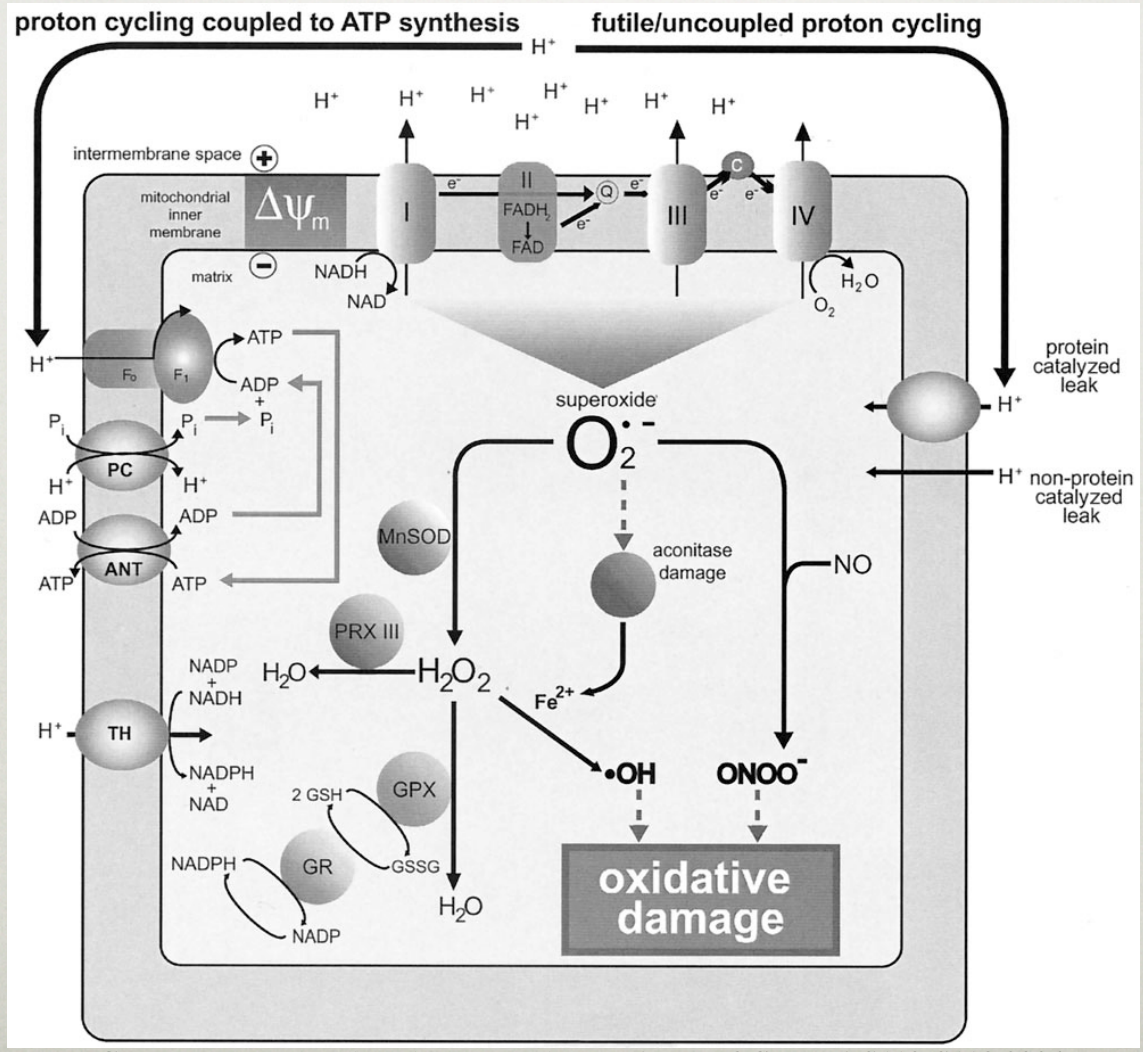
The mitochondrial genome:
mtDNA = 37 genes
Extracellular plasmids (chromosomes) ~ 1500 genes

Wallace DC. 2007.
Annu. Rev. Biochem. 76:781–821

mtDNA mutations

- Mutations → aging, mitochondrial dysfunction, diseases, cell death, etc.
- Mutation rate is 1–2 orders of magnitude higher than nuclear mutation rate.
- No recombination, so this high mutation rate is important in keeping mitochondria diverse, i.e., it is the adaptive engine.

Reactive Oxygen Species (ROS)



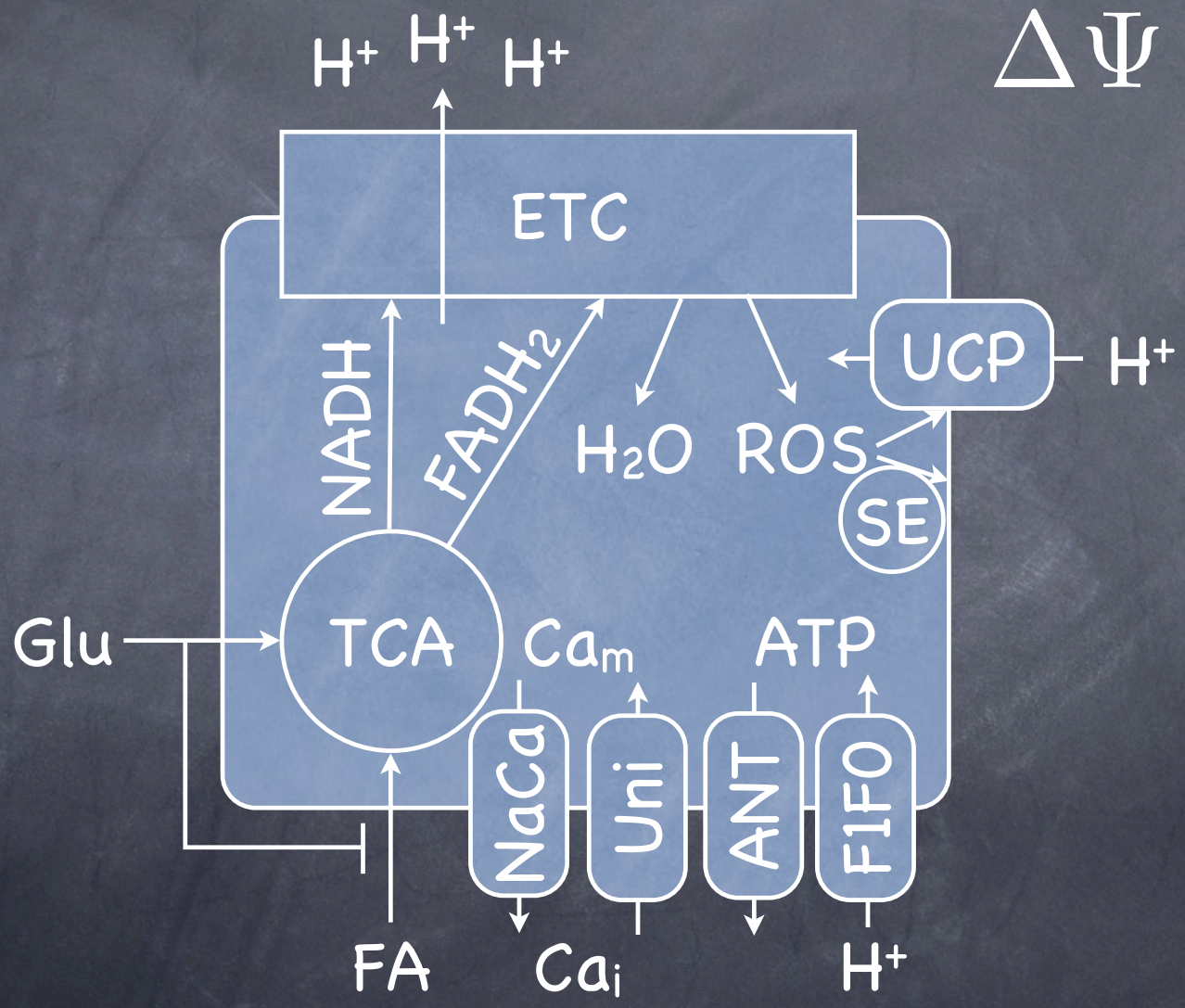
Green, K, MD Brand, and MP Murphy, *Diabetes*, **53 Suppl 1**:S110-S118 2004.

The Model

- Develop a mathematical model of respiration, ATP synthesis, and ROS production in response to glucose and fatty acid stimulation.

- R Bohnensack, J Bioenerg Biomembr, **14**:45-61, 1982.
- D Pietrobon and SR Caplan, Biochem, **24**:5764-5778, 1985.
- **G Magnus and J Keizer, Am J Physiol, 273:C717-C733, 1997, 274:C1158-C1173 and C1174-1184, 1998.**
  - S Cortassa et al., Biophys J, **84**:2734-2755, 2003.
  - R Bertram et al., J Theor Biol, **243**:575-586, 2006.
- S Salinari et al., Am J Physiol Endocrinol Metab, **293**:E396-E409, 2007.

The Model

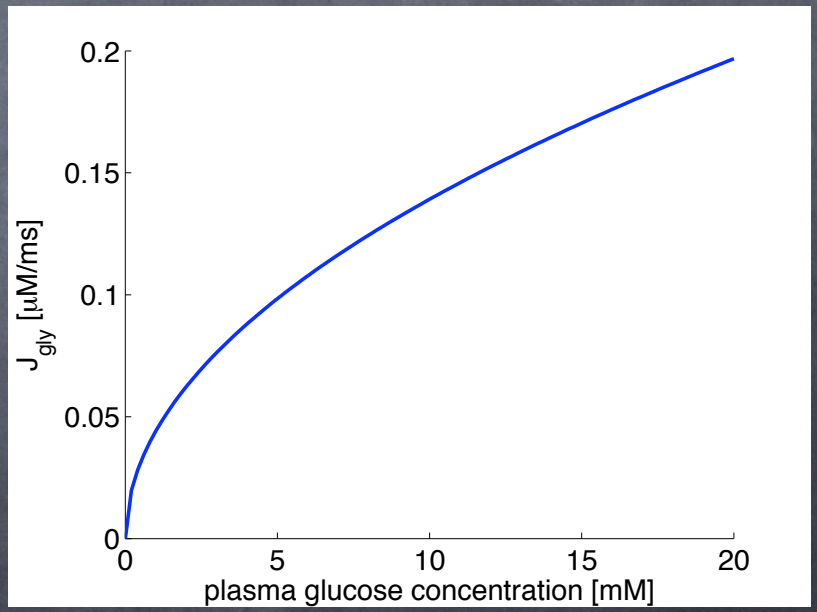

H+ H+ H+
ΔΨ
ETC
NADH
FADH2
H2O
ROS
UCP
H+
SE
Glu
TCA
Cam
ATP
NaCa
Uni
ANT
F1FO
FA
Cai
H+

The Mitochondria

Glucose Input

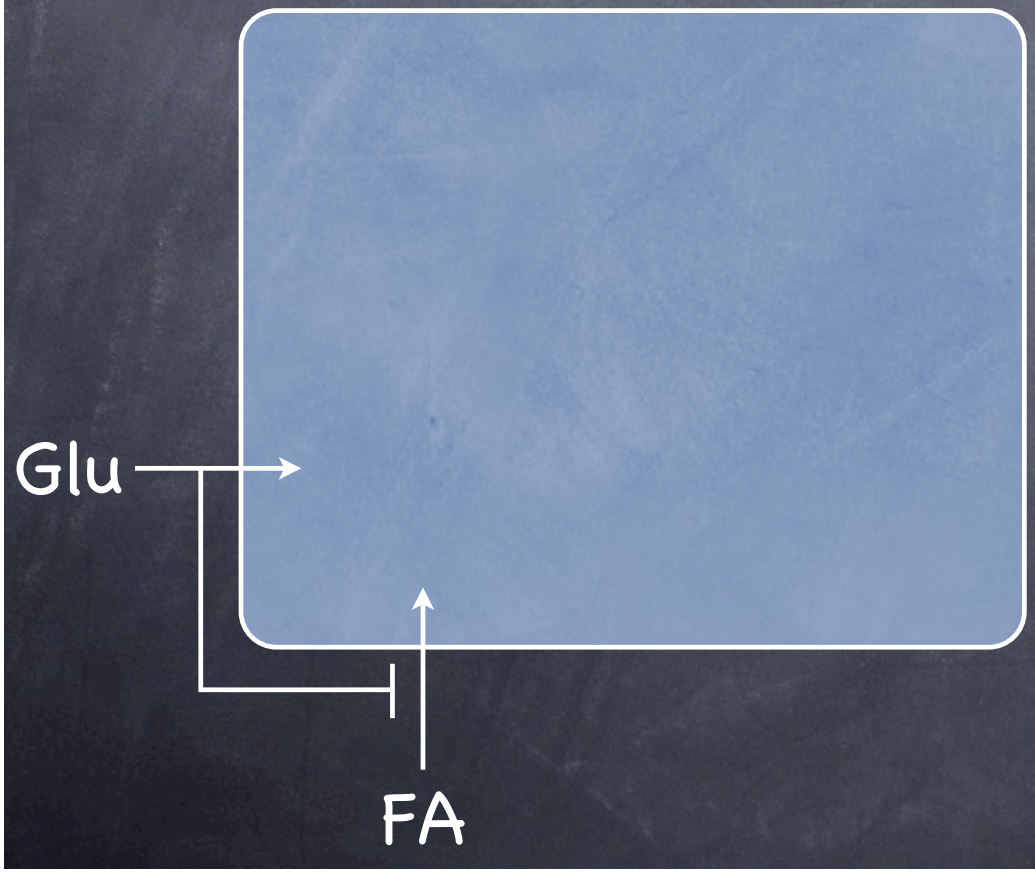
$$J_{gly} = p_1 \sqrt{\frac{Glu}{1\mathrm{mM}}}$$

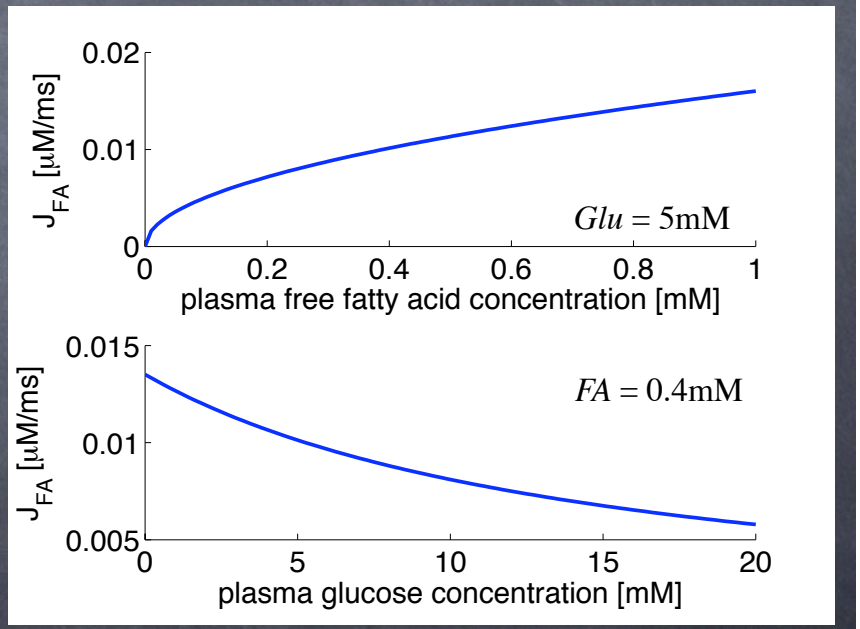
Glu →



Fatty Acid (Palmitate) Input

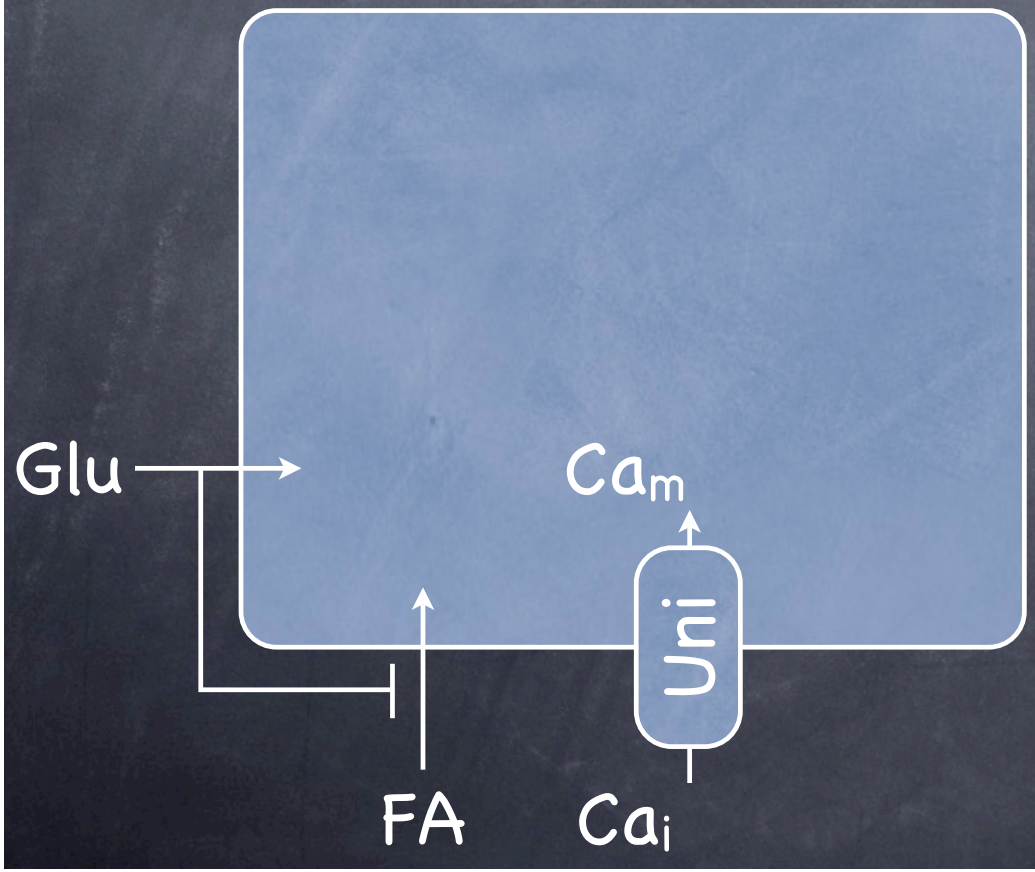
$$J_{FA} = \frac{p_2}{Glu + p_3}\sqrt{\frac{FA}{1\text{mM}}}$$

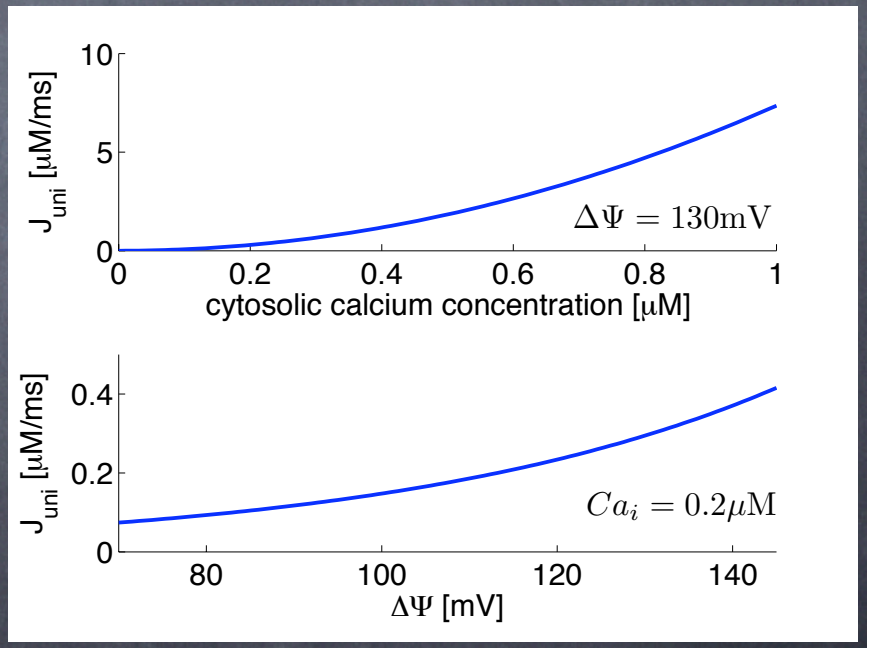




Calcium Uniporter

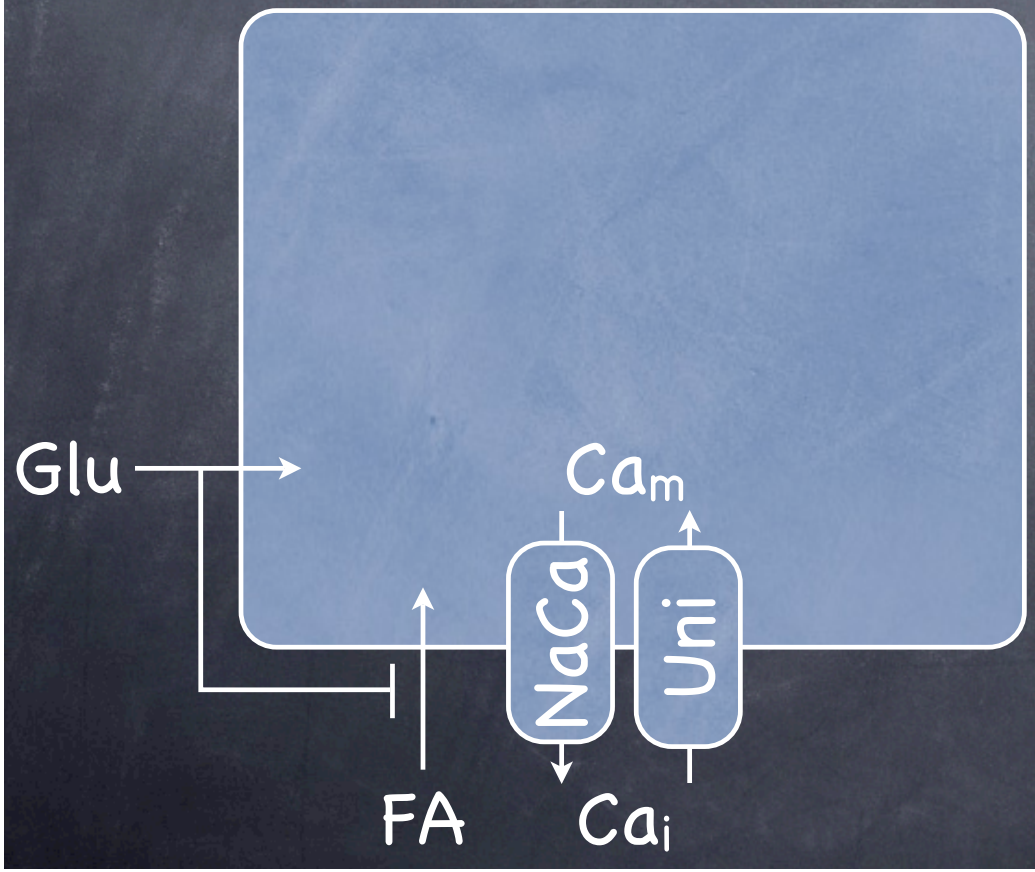
$$J_{uni} = p_4 e^{p_5 \Delta\Psi} \frac{Ca_i^2}{1\mu\mathrm{M}^2}$$

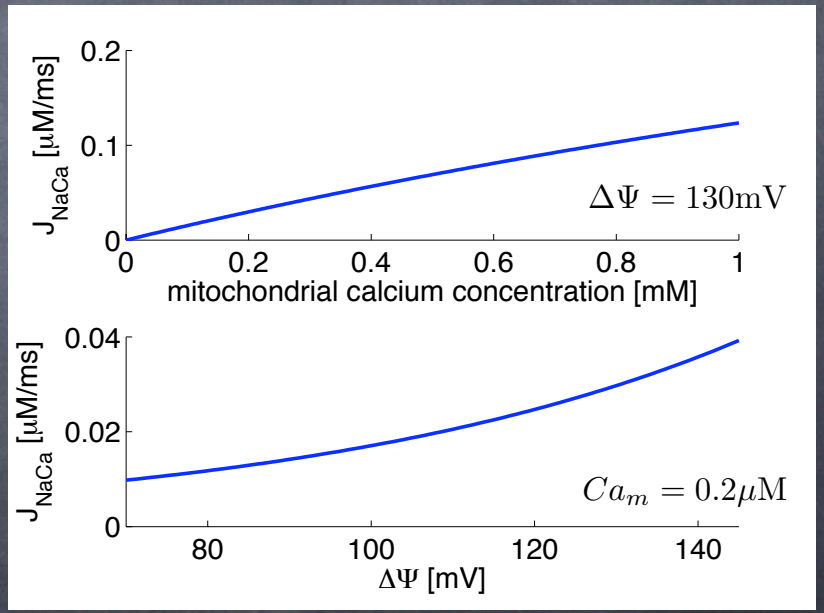




Sodium/Calcium Exchanger

$$J_{NaCa} = \frac{p_6 Ca_m}{p_7 + Ca_m} e^{p_8 \Delta\Psi}$$

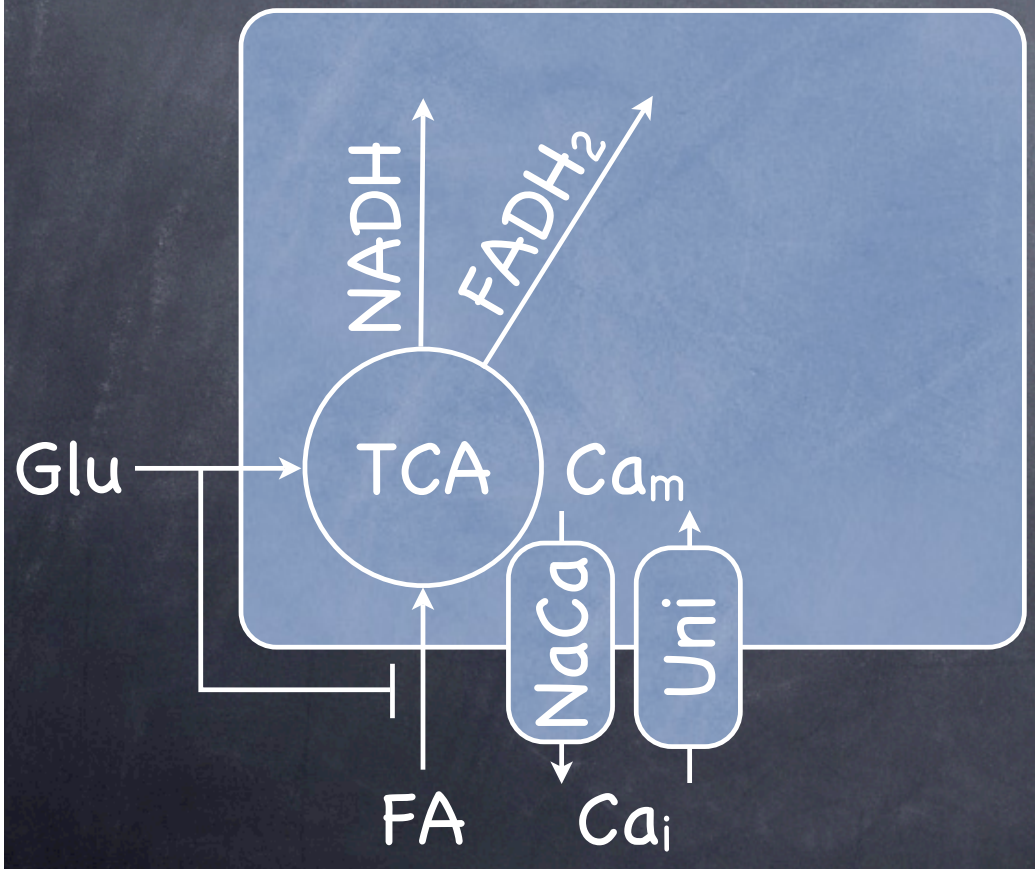


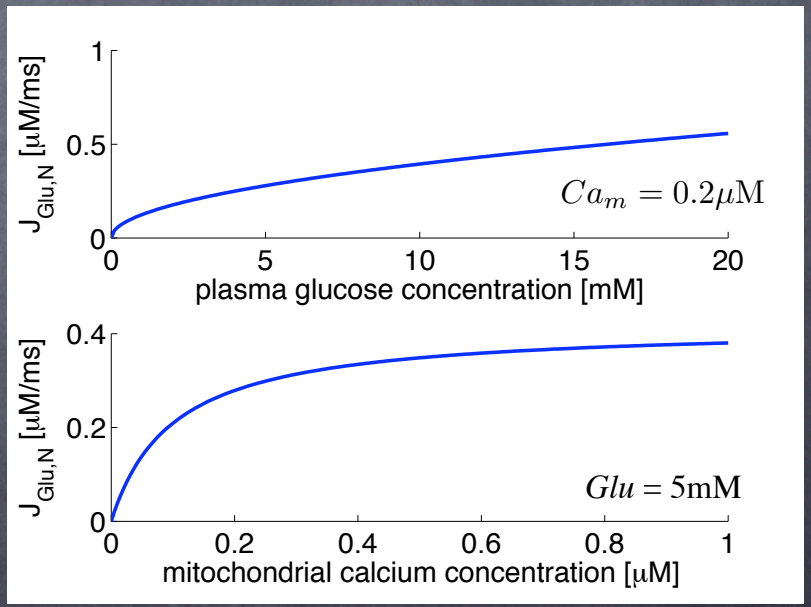


NADH and $FADH_2$ Production from Glucose

$$J_{Glu,N} = p_9 J_{gly}\left(\frac{Ca_m}{p_{10}+Ca_m}\right)$$

$$J_{Glu,F} = p_{11} J_{gly}\left(\frac{Ca_m}{p_{10}+Ca_m}\right)$$

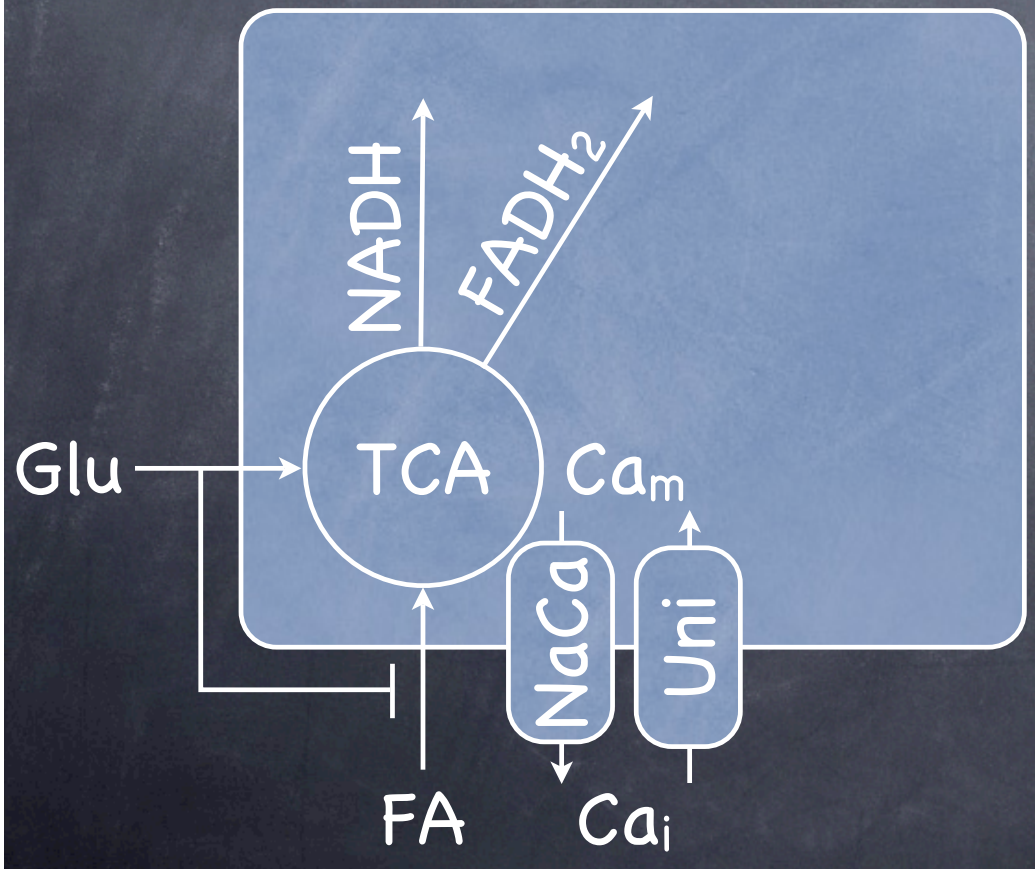




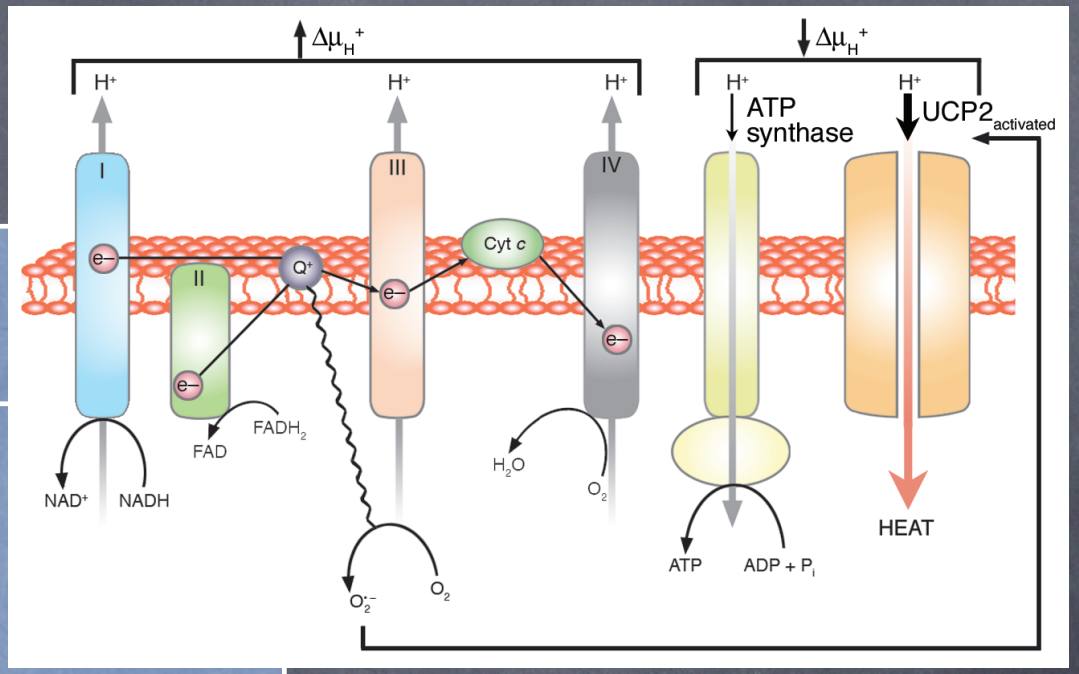
NADH and $FADH_2$ Production from Fatty Acids

$$J_{FA,N} = p_{12} J_{FA} \left(\frac{Ca_m}{p_{10} + Ca_m} \right).$$

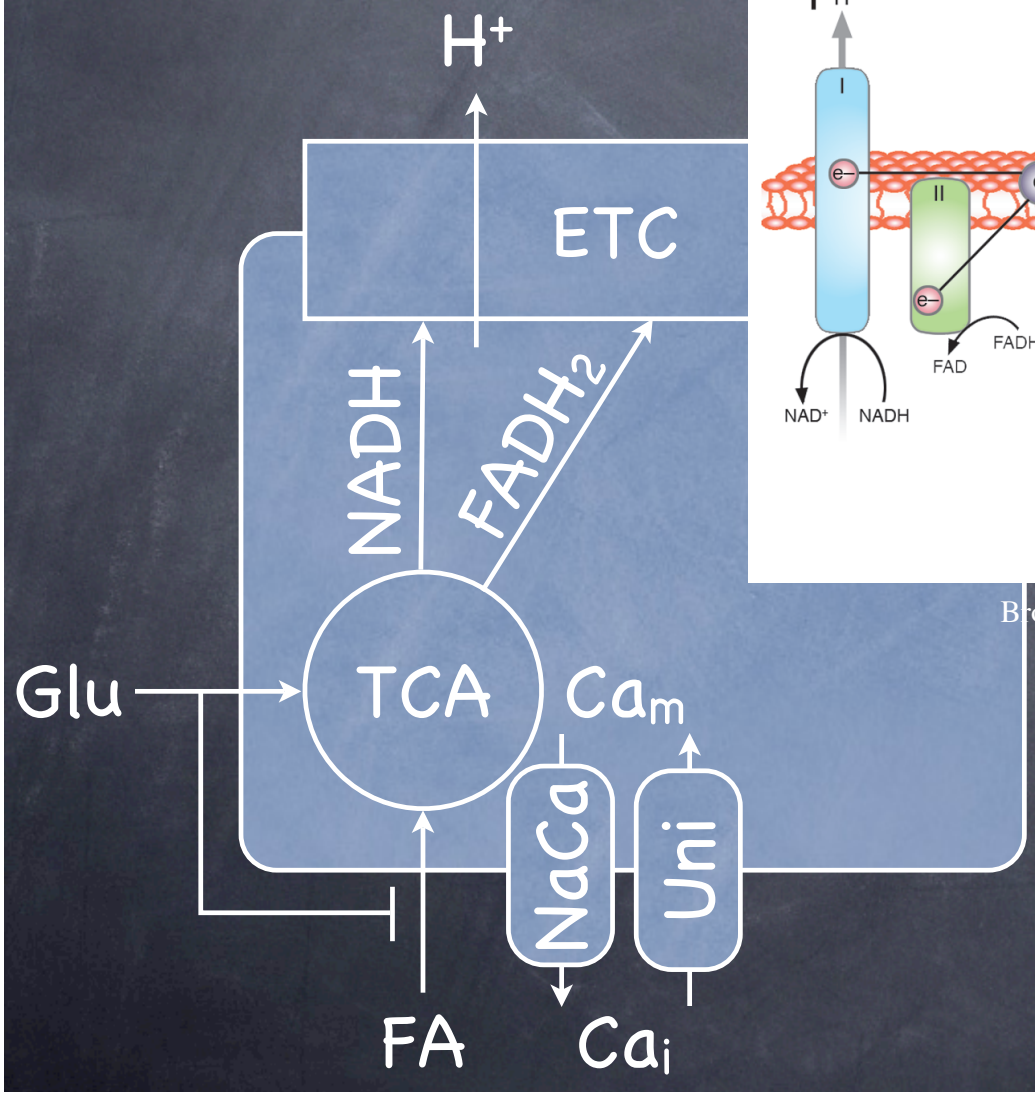
$$J_{FA,F} = p_{13} J_{FA} \left(\frac{Ca_m}{p_{10} + Ca_m} \right).$$



Electron Transport Chain (ETC)



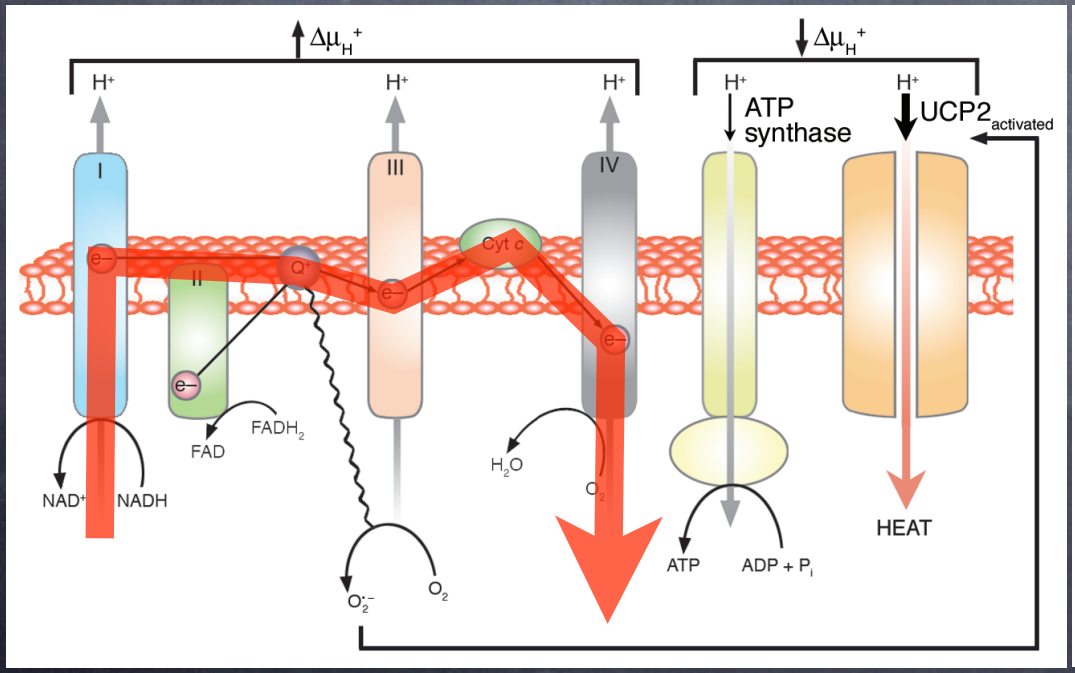
Brownlee, *J Clin Invest*, **112**:1788-1790, 2003.

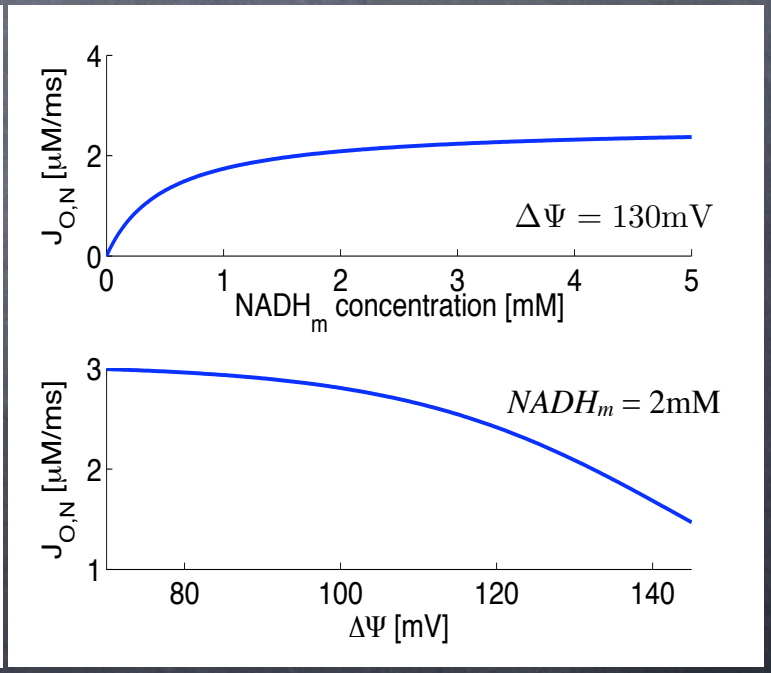


NADH Oxidation

$$J_{O,N} = \frac{p_{14} NADH_m}{p_{15} + NADH_m} \left(\frac{1}{1 + e^{(\Delta\Psi - p_{16})/p_{17}}} \right)$$

$$J_{Hres,N} = \frac{p_{18} NADH_m}{p_{15} + NADH_m} \left(\frac{1}{1 + e^{(\Delta\Psi - p_{16})/p_{17}}} \right)$$



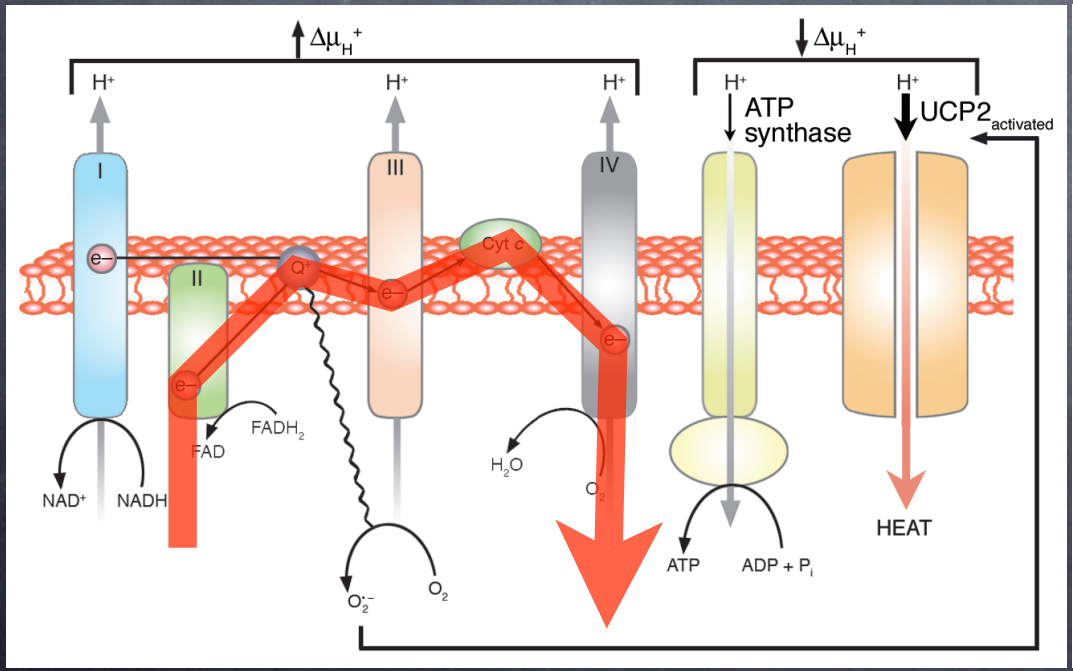


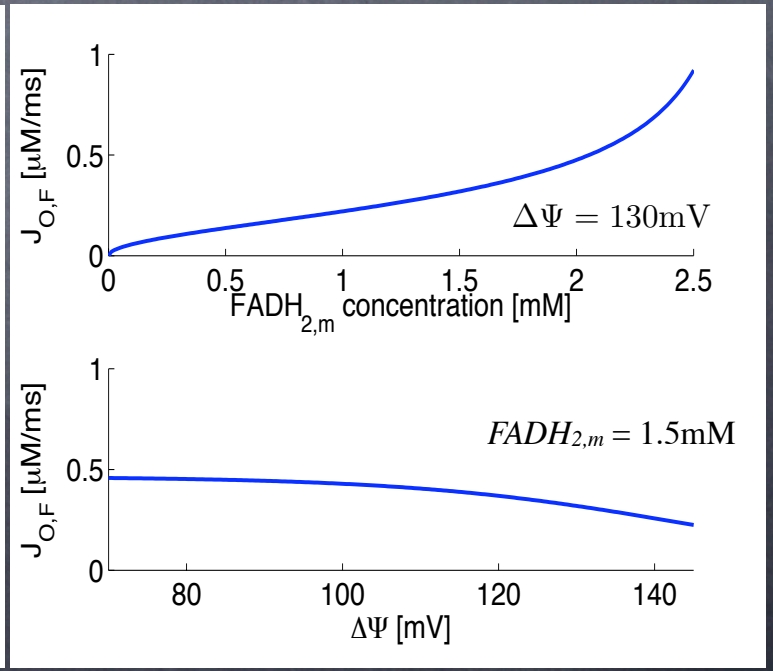
Brownlee, *J Clin Invest*, **112**:1788-1790, 2003.

$FADH_2$ Oxidation

$$J_{O,F} = p_{19}\sqrt{\frac{FADH_{2,m}}{FAD_m}}\left(\frac{1}{1+e^{(\Delta\Psi - p_{20})/p_{21}}}\right)$$

$$J_{Hres,F} = p_{22}\sqrt{\frac{FADH_{2,m}}{FAD_m}}\left(\frac{1}{1+e^{(\Delta\Psi - p_{20})/p_{21}}}\right).$$



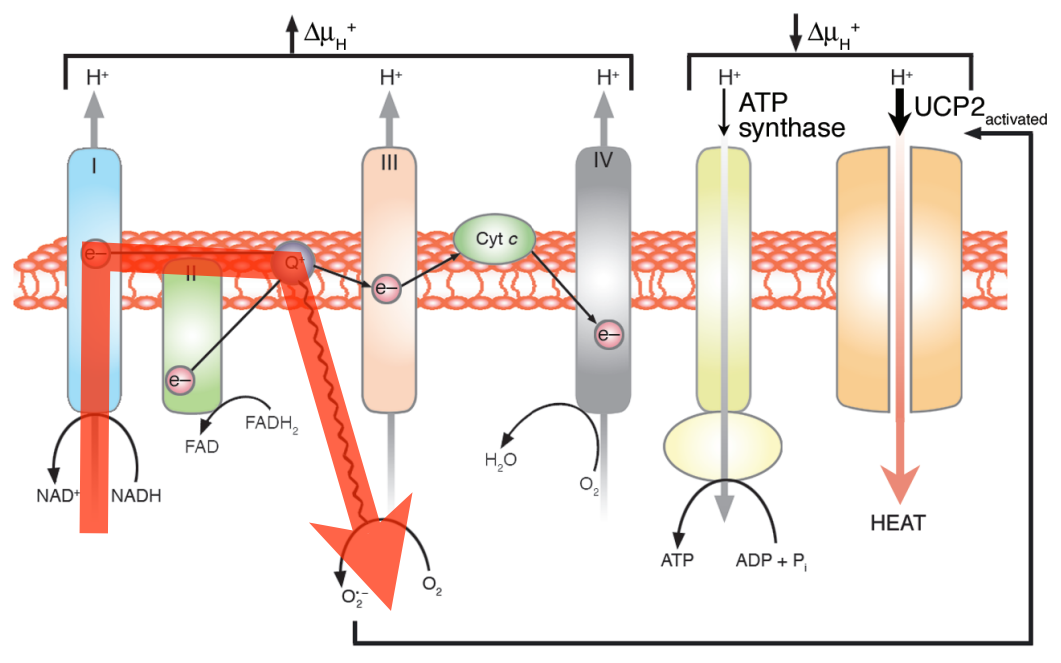


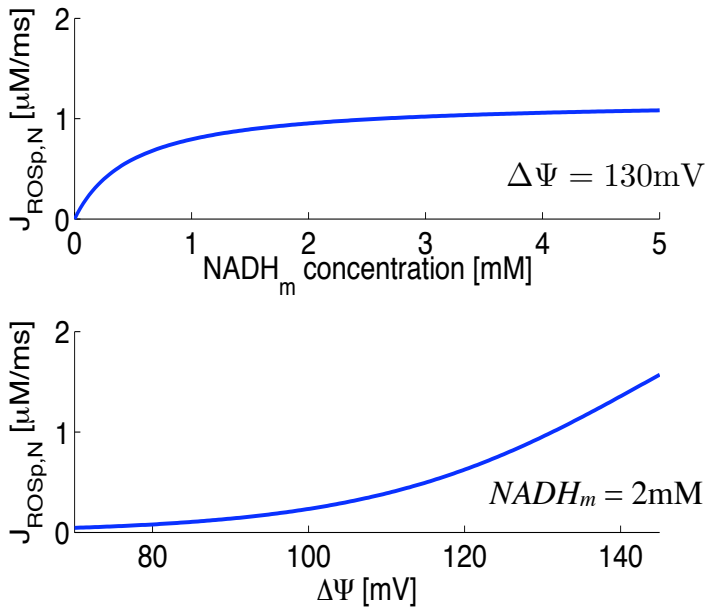
Brownlee, *J Clin Invest*, **112**:1788-1790, 2003.

ROS Production from NADH Oxidation

$$J_{ROSp,N} = \frac{p_{14} NADH_m}{p_{15} + NADH_m} \left(1 - \frac{1}{1 + e^{(\Delta\Psi - p_{16})/p_{17}}}\right)$$

$$J_{Hros,N} = p_{23} \frac{p_{18} NADH_m}{p_{15} + NADH_m} \left(1 - \frac{1}{1 + e^{(\Delta\Psi - p_{16})/p_{17}}}\right)$$



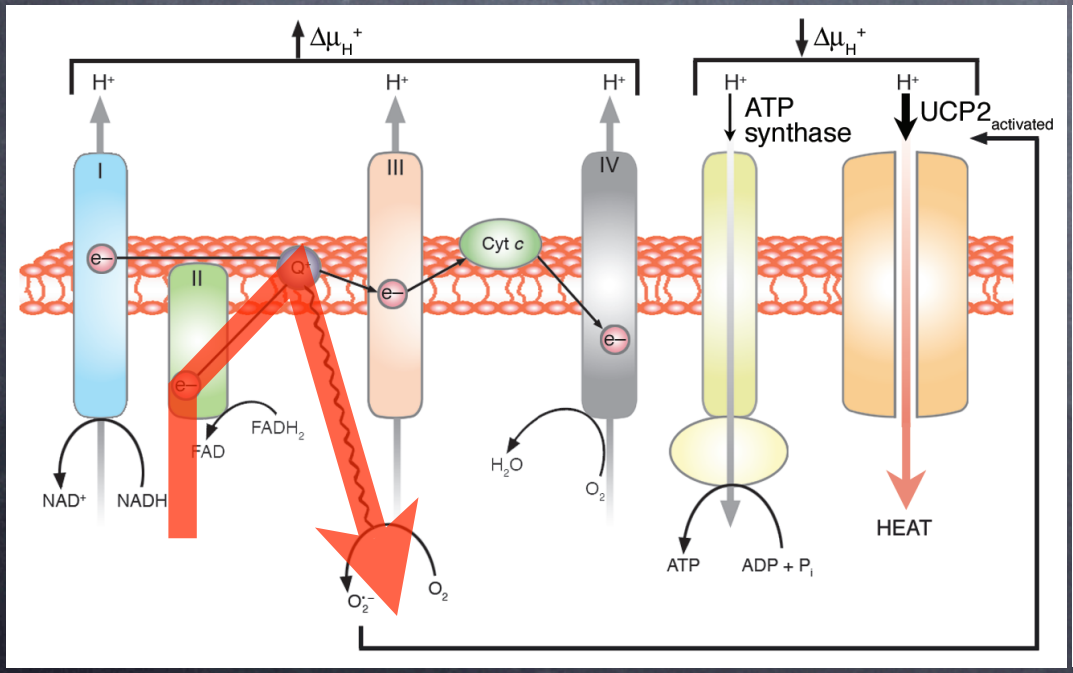


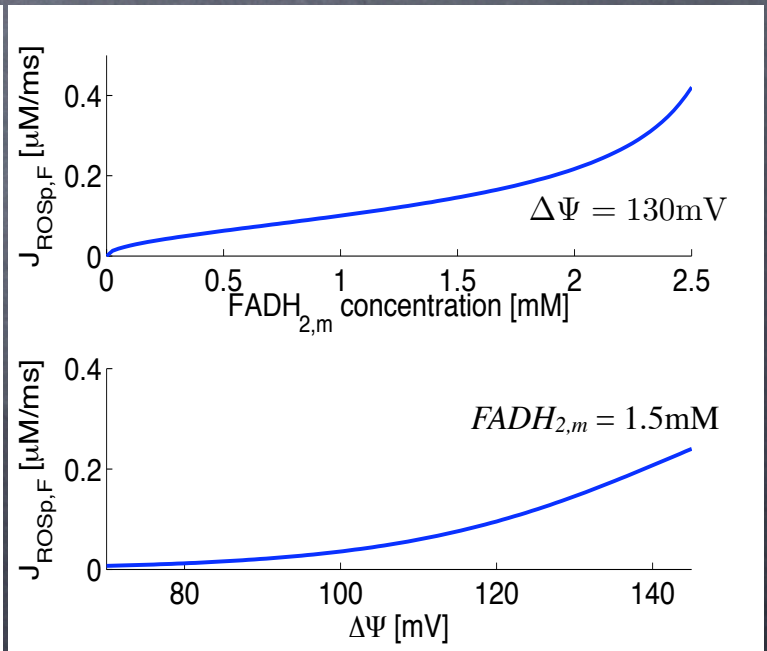
Brownlee, *J Clin Invest*, **112**:1788-1790, 2003.

ROS Production from $FADH_2$ Oxidation

$$J_{ROSp,F} = p_{19}\sqrt{\frac{FADH_{2,m}}{FAD_m}}\left(1 - \frac{1}{1 + e^{(\Delta\Psi - p_{20})/p_{21}}}\right)$$

$$J_{Hros,F} = p_{23}p_{22}\sqrt{\frac{FADH_{2,m}}{FAD_m}}\left(1 - \frac{1}{1 + e^{(\Delta\Psi - p_{20})/p_{21}}}\right)$$



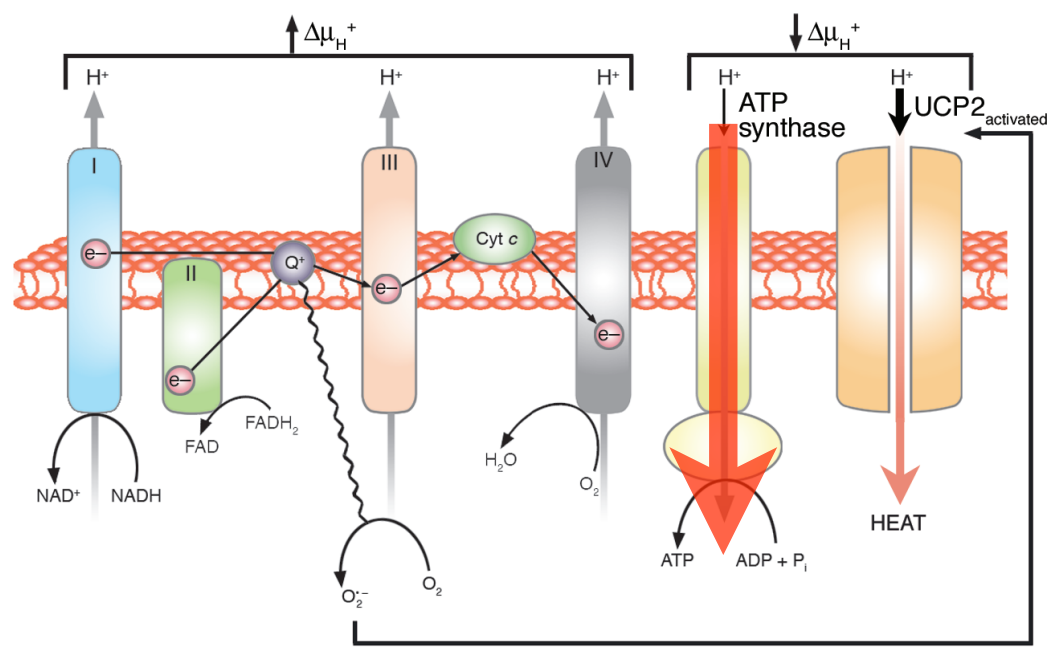


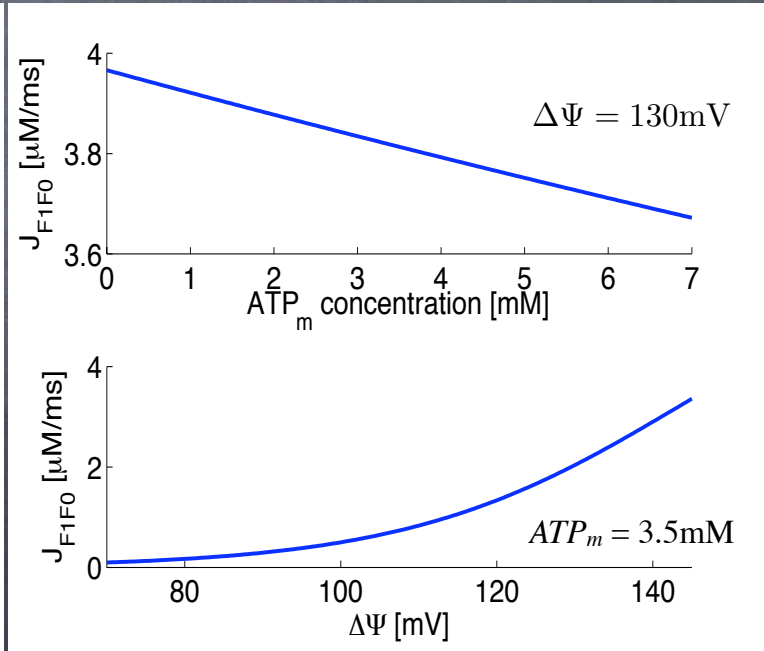
Brownlee, *J Clin Invest*, **112**:1788-1790, 2003.

ATP Synthesis

$$J_{F1F0} = \frac{p_{26}}{p_{27} + ATP_m}\left(\frac{1}{1 + e^{(p_{28} - \Delta\Psi)/p_{29}}}\right)$$

$$J_{H,atp} = \frac{p_{30}}{p_{27} + ATP_m}\left(\frac{1}{1 + e^{(p_{28} - \Delta\Psi)/p_{29}}}\right)$$





Brownlee, *J Clin Invest*, **112**:1788-1790, 2003.

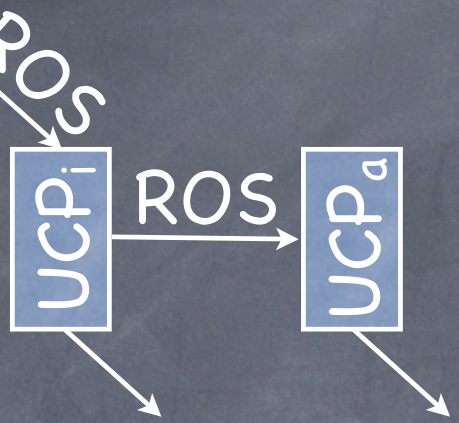
Uncoupling Proteins (UCP)

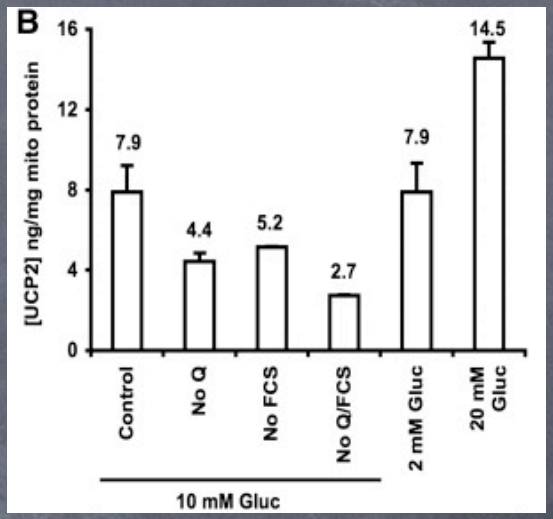
$$J_{UCP,p} = p_{37} ROS$$

$$J_{UCP,a} = p_{38} UCP_i ROS$$

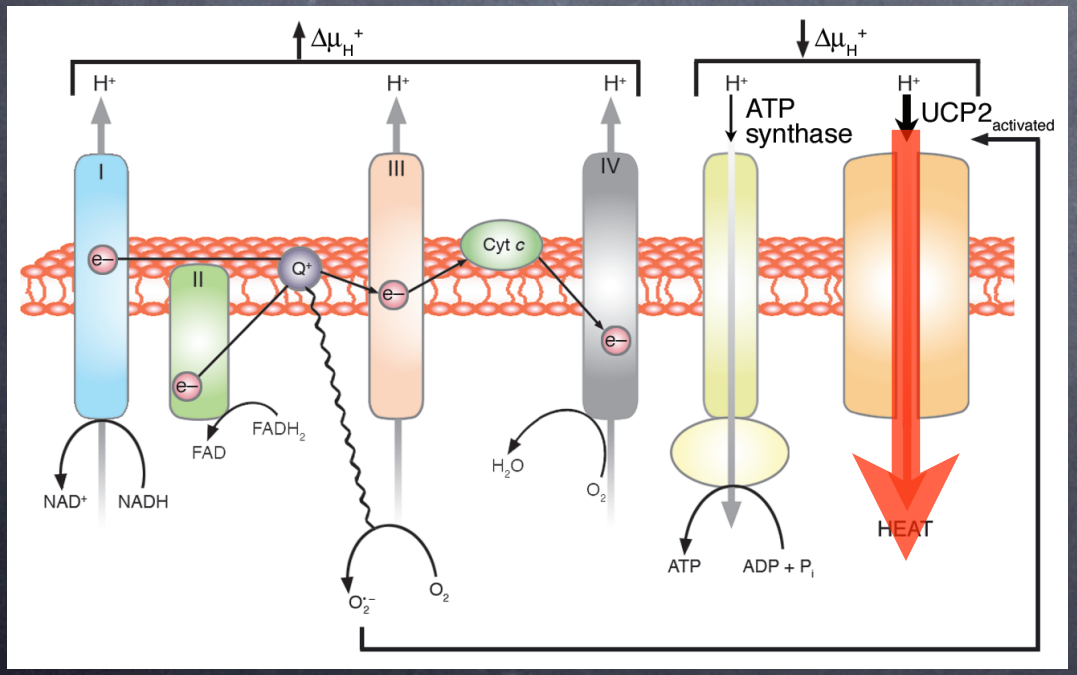
$$J_{UCP,d} = p_{39} \left(UCP_i - UCP_o \right).$$

$$J_{UCP,i} = p_{40} UCP_a$$

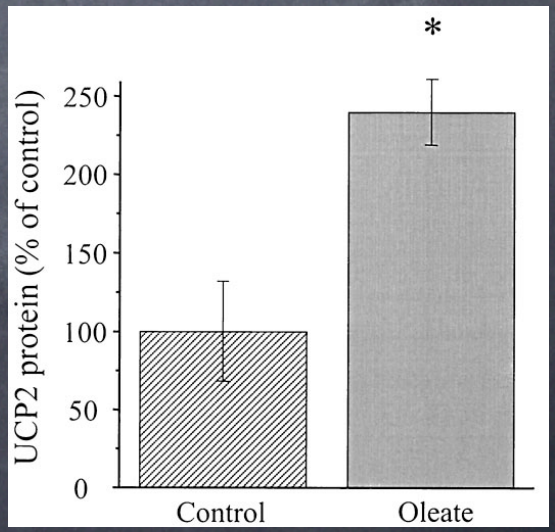




Azzu *et al.*, *Biochim. Biophys. Acta*, **1777**:1378-1383, 2008.



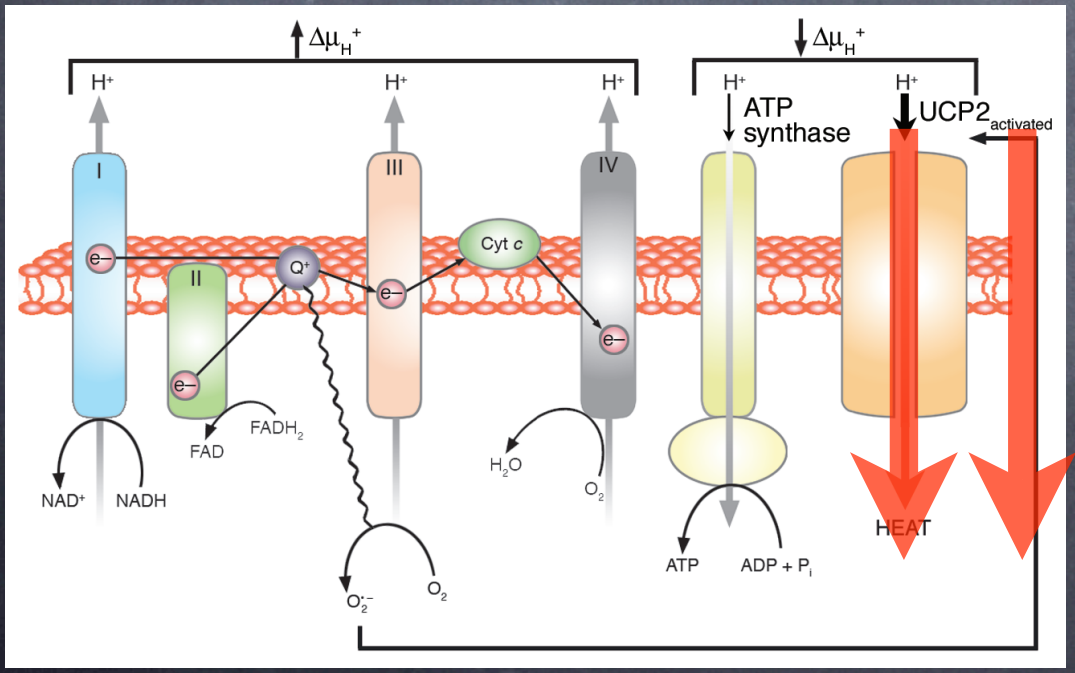
Brownlee, *J Clin Invest*, **112**:1788-1790, 2003.



Lameloise *et al.*, *Diabetes*, **50**:803-809, 2001.

Proton Leak

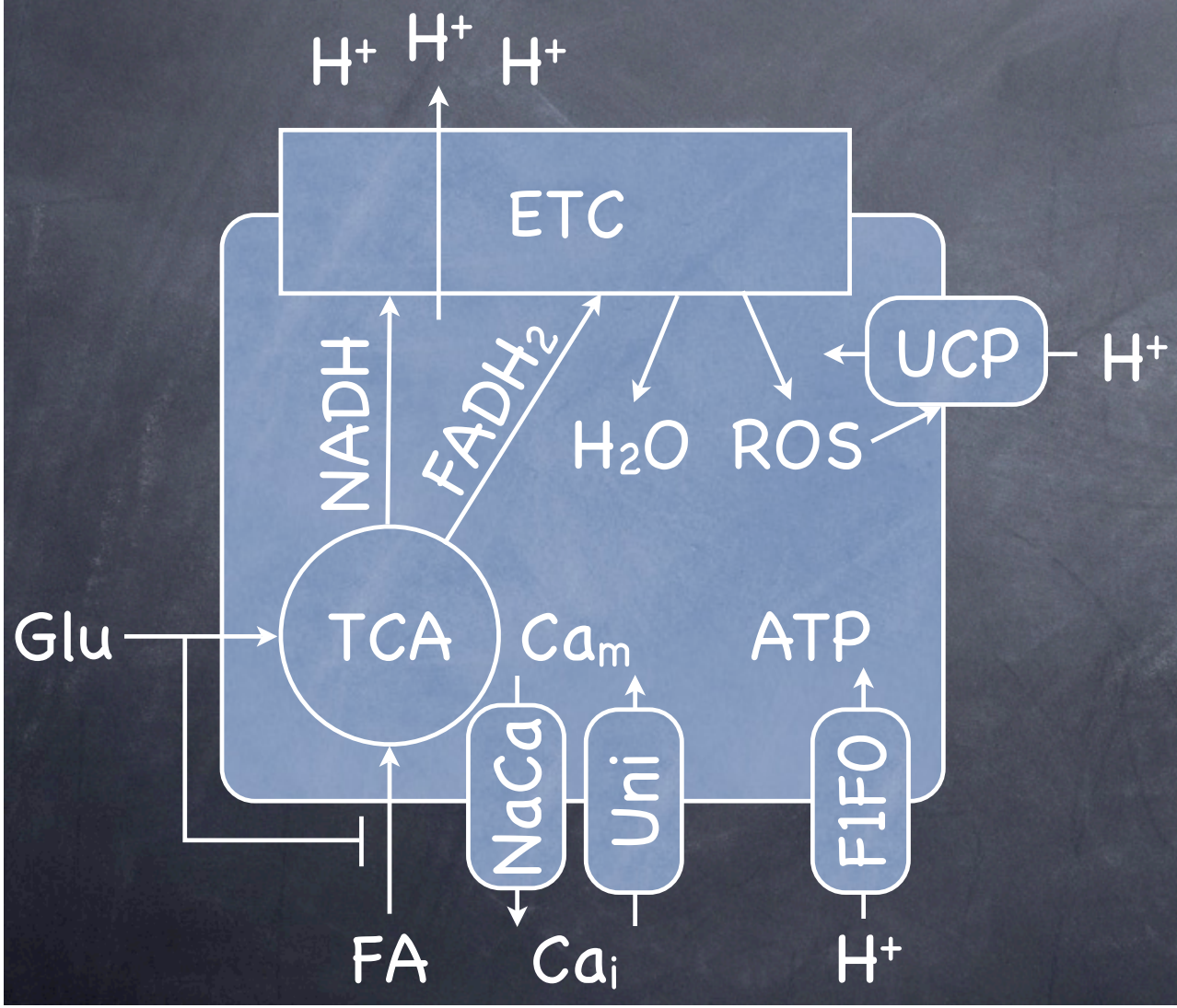
$$J_{H,leak} = p_{41}\left(\Delta\Psi + p_{42}\right) + p_{43}UCP_a$$



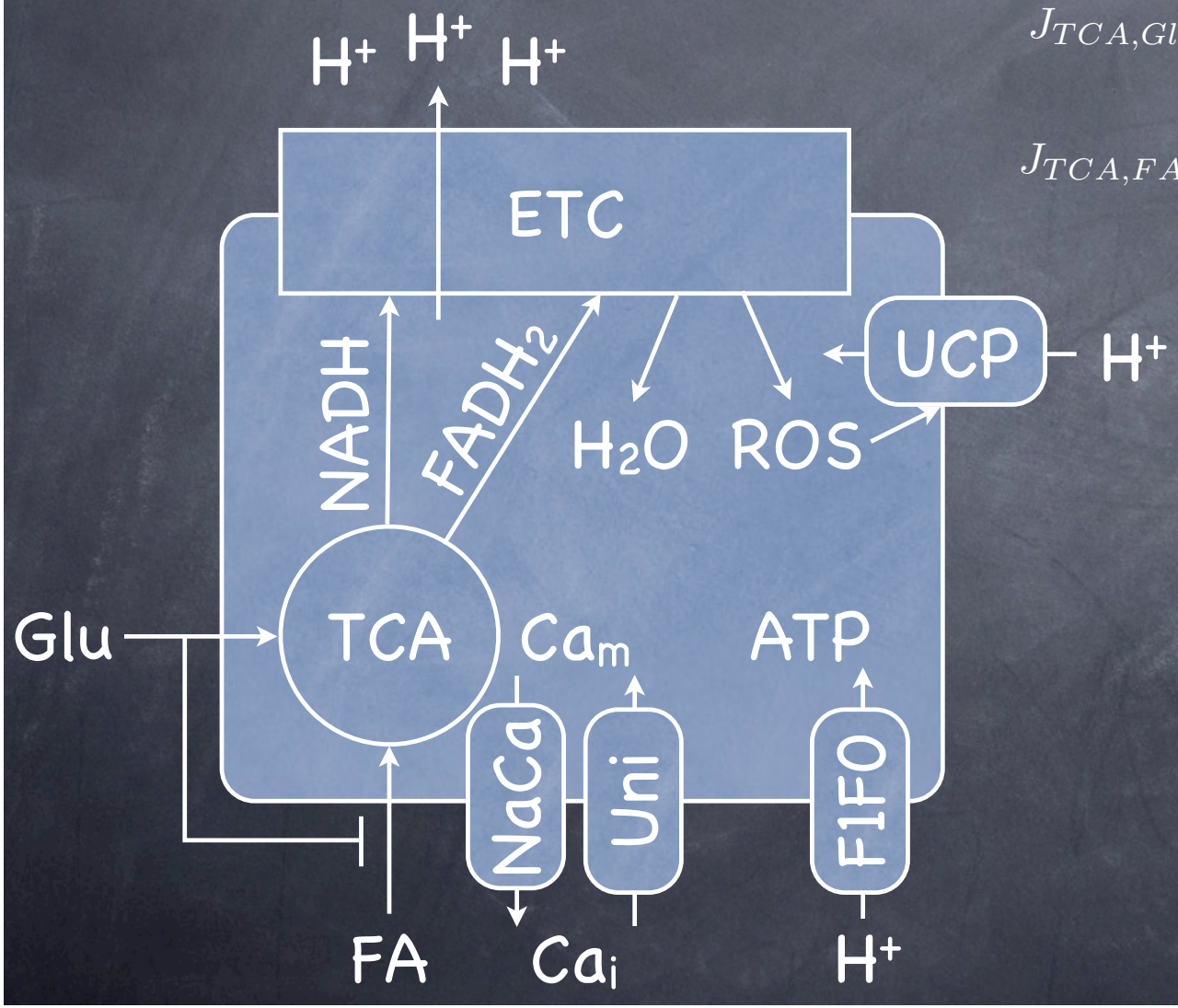
$\Delta\Psi$

Brownlee, *J Clin Invest*, **112**:1788-1790, 2003.

So we've added:



Additional ATP Production

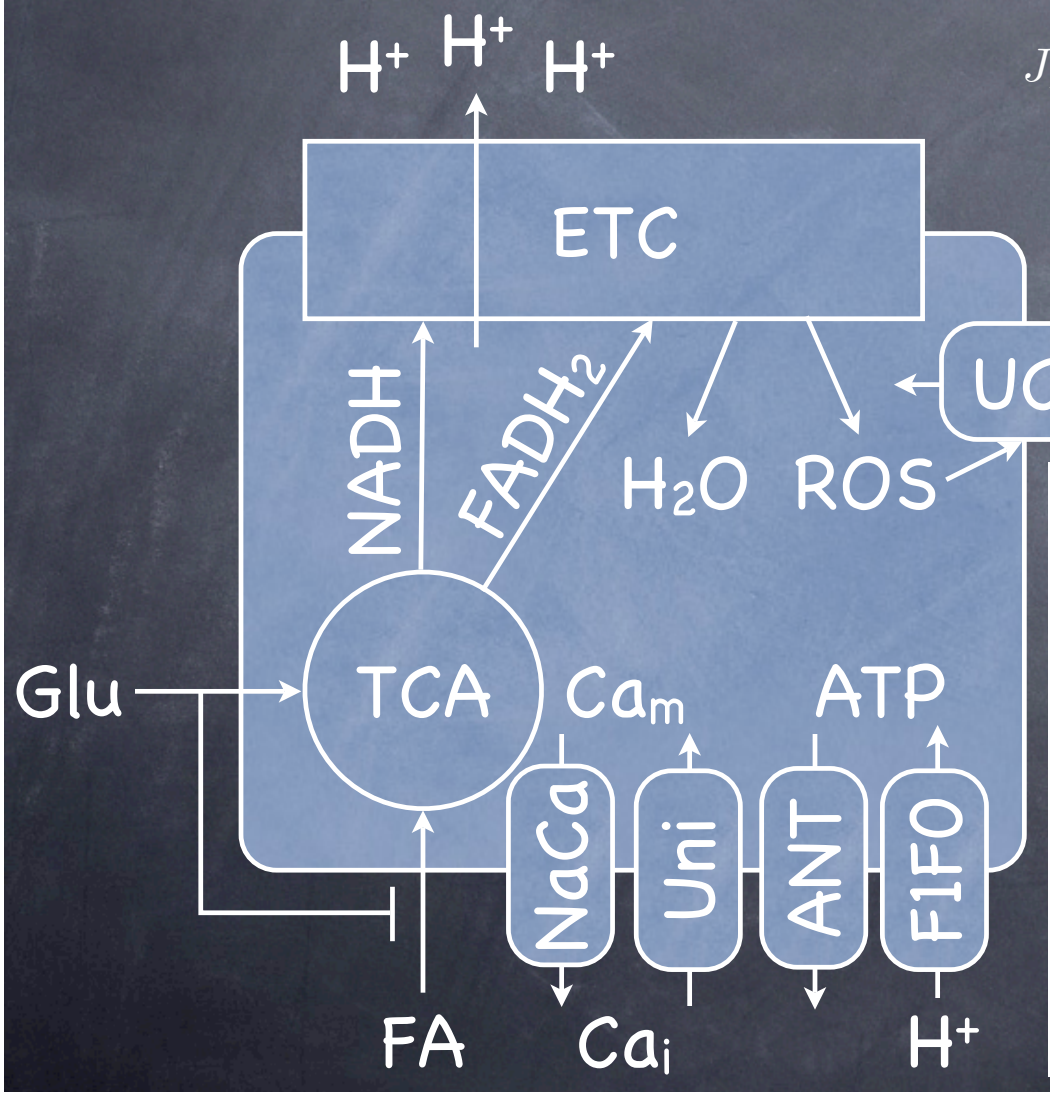


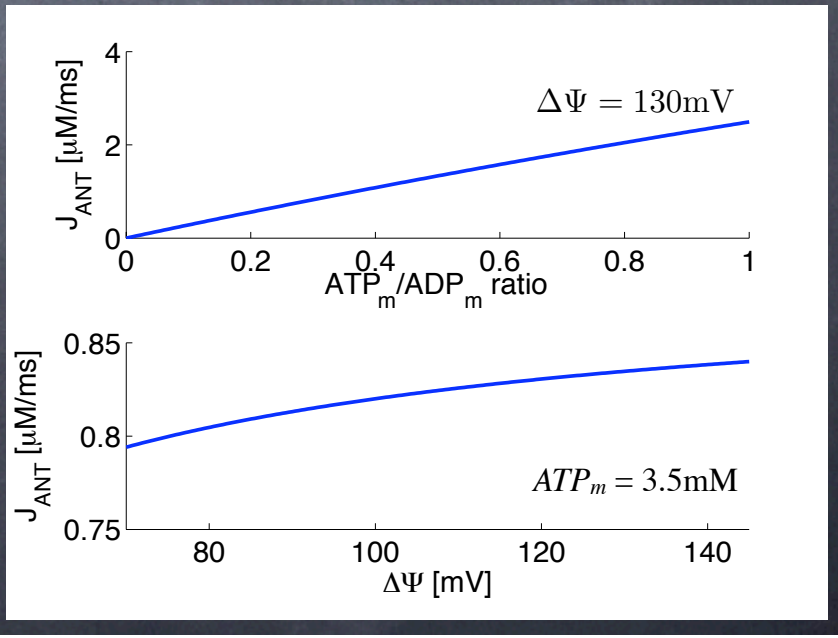
$$J_{TCA,Glu} = p_{24} J_{gly} \left(\frac{Ca_m}{p_{10} + Ca_m} \right)$$

$$J_{TCA,FA} = p_{25} J_{FA} \left(\frac{Ca_m}{p_{10} + Ca_m} \right).$$

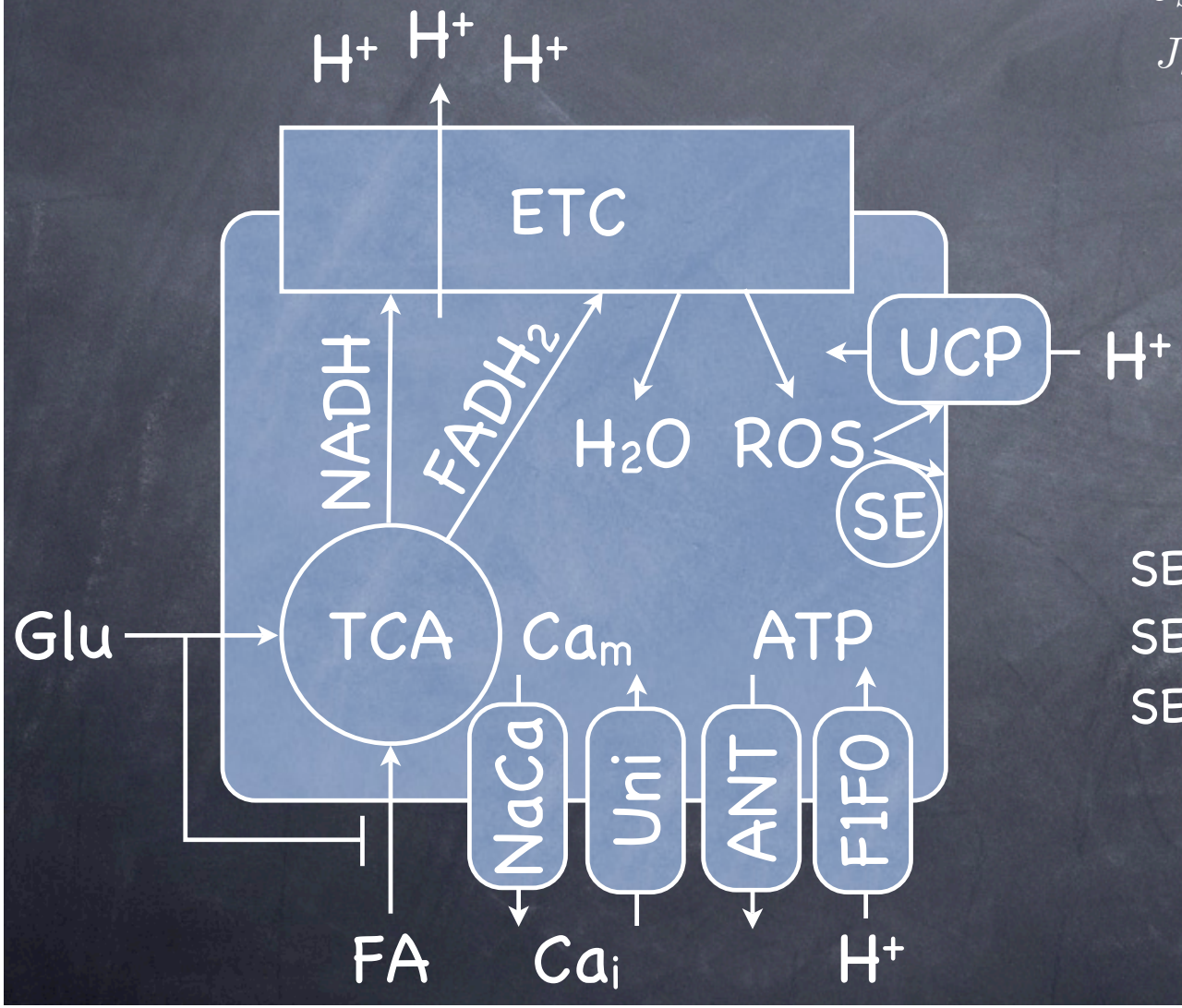
The Adenine Nucleotide Translocator

$$J_{ANT} = \frac{p_{31} ATP_m}{ATP_m + p_{32} ADP_m} \left(\frac{\Delta\Psi}{\Delta\Psi + p_{33}} \right)$$





Scavenging Enzymes



$$J_{SE,3} = p_{34} SE_3 ROS$$

$$J_{SE,i} = p_{35} SE_2 ROS$$

$$J_{SC,a} = p_{36} SE_x$$

$SE_3 + ROS \longrightarrow SE_2$

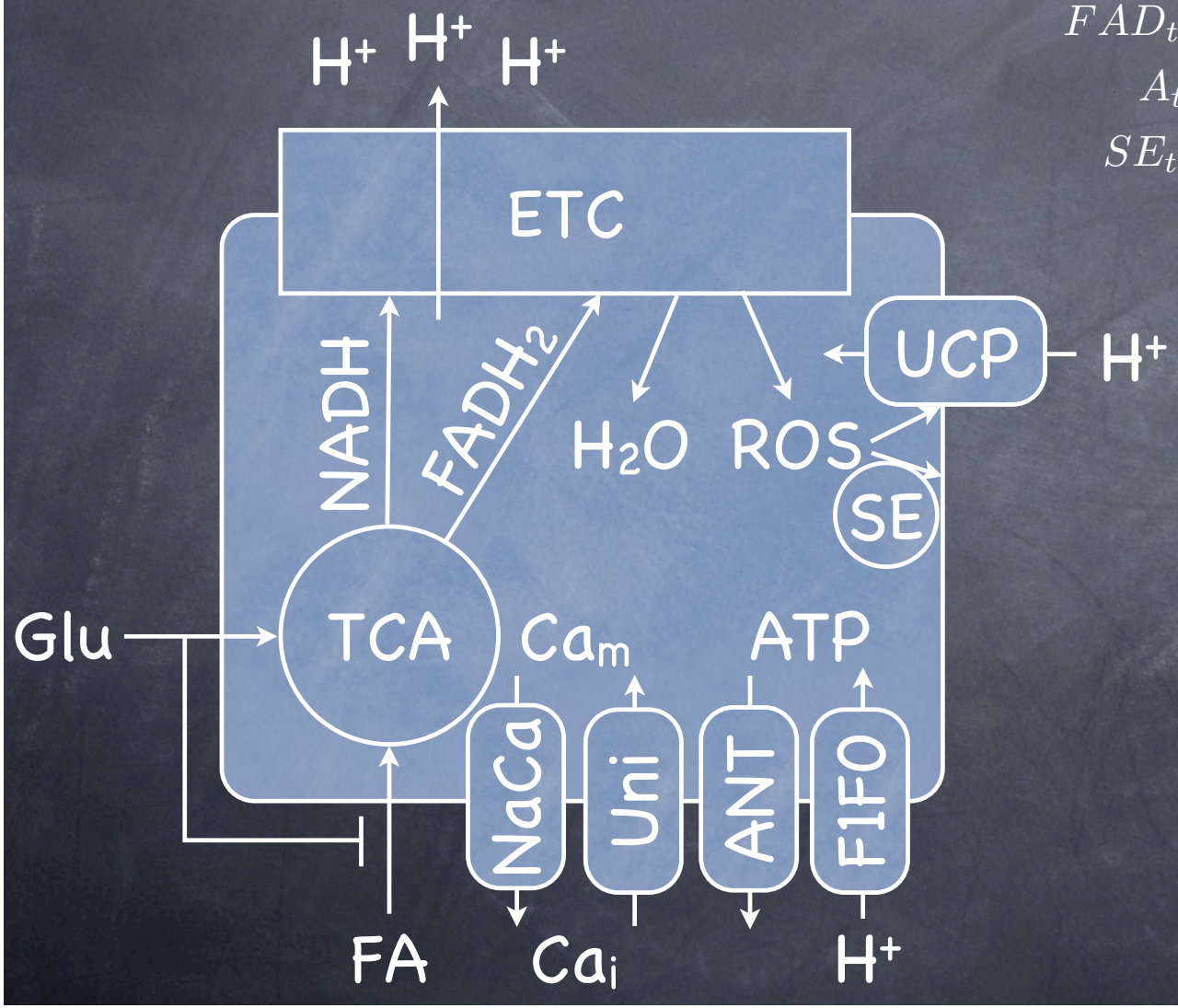
$SE_2 + ROS \longrightarrow SE_3$

$SE_2 + ROS \longrightarrow SE_x$

$SE_x \longrightarrow SE_3$

Conserved Quantities

$$NAD_{tot} = NAD_m + NADH_m$$
$$FAD_{tot} = FAD_m + FADH_{2,m}$$
$$A_{tot} = ADP_m + ATP_m$$
$$SE_{tot} = SE_3 + SE_2 + SE_x$$



The Complete Model

$$\frac{dNADH_m}{dt} = \gamma\left(J_{Glu,N} + J_{FA,N} - J_{O,N} - J_{ROSp,N}\right)$$

$$\frac{dFADH_{2,m}}{dt} = \gamma\left(J_{Glu,F} + J_{FA,F} - J_{O,F} - J_{ROSp,F}\right)$$

$$\frac{dADP_m}{dt} = \gamma\left(J_{ANT} - J_{F1F0} - J_{TCA,Glu} - J_{TCA,FA}\right)$$

$$\frac{dCa_m}{dt} = f_m\left(J_{uni} - J_{NaCa}\right)$$

$$\frac{d\Delta\Psi}{dt} = \left(J_{Hres,N} + J_{Hres,F} + J_{Hros,N} + J_{Hros,F} - J_{H,atp} - J_{ANT} - J_{H,leak} - J_{NaCa} - 2J_{uni}\right)/C_m$$

$$\frac{dROS}{dt} = 2\left(J_{ROSp,N} + J_{ROSp,F}\right) - J_{SE,3} - 2J_{SE,i} - J_{UCP,a}$$

$$\frac{dSE_3}{dt} = J_{SE,i} + J_{SE,a} - J_{SE,3}$$

$$\frac{dSE_x}{dt} = J_{SE,i} - J_{SE,a}$$

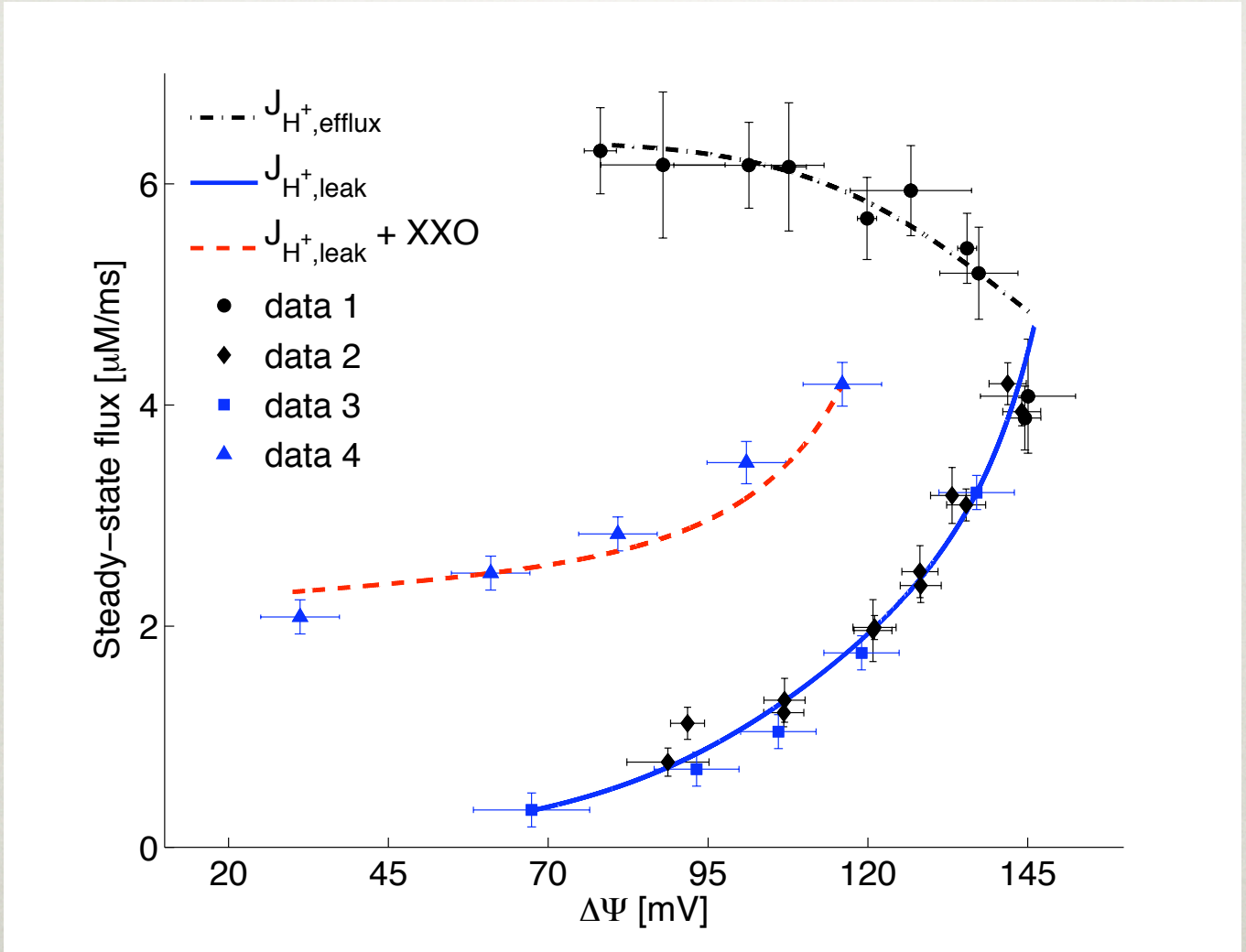
$$\frac{dUCP_i}{dt} = J_{UCP,p} - J_{UCP,a} - J_{UCP,d}$$

$$\frac{dUCP_a}{dt} = J_{UCP,a} - J_{UCP,i}$$

Parameter	Value	Units
γ	0.001	mM μM^{-1}
f_m	0.0003	unitless
C_m	1.8	μM mV^{-1}
NAD_{tot}	10	mM
FAD_{tot}	2.75	mM
A_{tot}	15	mM
SE_{tot}	2	μM
p_1	0.044	μM ms^{-1}
p_2	0.32	μM mM ms^{-1}
p_3	14.98	mM
p_4	0.37	μM^{-1} ms^{-1}
p_5	0.023	mV^{-1}
p_6	0.053	μM ms^{-1}
p_7	3.752	μM
p_8	0.0185	mV^{-1}
p_9	4.25	unitless
p_{10}	0.1	μM
p_{11}	2.125	unitless
p_{12}	16.4688	unitless
p_{13}	7.9688	unitless
p_{14}	3.8	μM ms^{-1}
p_{15}	0.5	mM
p_{16}	143.8274	mV
p_{17}	17.6353	mV
p_{18}	45.6	μM ms^{-1}
p_{19}	0.4242	μM ms^{-1}
p_{20}	17.6353	mV
p_{21}	143.8274	mV
p_{22}	3.3936	μM ms^{-1}
p_{23}	0.5	unitless
p_{24}	1.06	unitless
p_{25}	4.25	unitless
p_{26}	590.7	μM mM ms^{-1}
p_{27}	87.3624	mM
p_{28}	17.6353	mV
p_{29}	143.8274	mV
p_{30}	1772.1	μM mM ms^{-1}
p_{31}	20.2584	μM ms^{-1}
p_{32}	6.6409	unitless
p_{33}	8.2612	mV
p_{34}	1.5	μM^{-1} ms^{-1}
p_{35}	0.55	μM^{-1} ms^{-1}
p_{36}	0.117	ms^{-1}
p_{37}	8×10^{-8}	ms^{-1}
p_{38}	2.8293×10^{-6}	μM^{-1} ms^{-1}
p_{39}	1.9254×10^{-7}	ms^{-1}
p_{40}	1.9254×10^{-7}	ms^{-1}
p_{41}	0.004	μM mV^{-1} ms^{-1}
p_{42}	-25	mV
p_{43}	7.6176	ms^{-1}
UCP_o	0.25	μM

Non-Ohmic Proton Leak

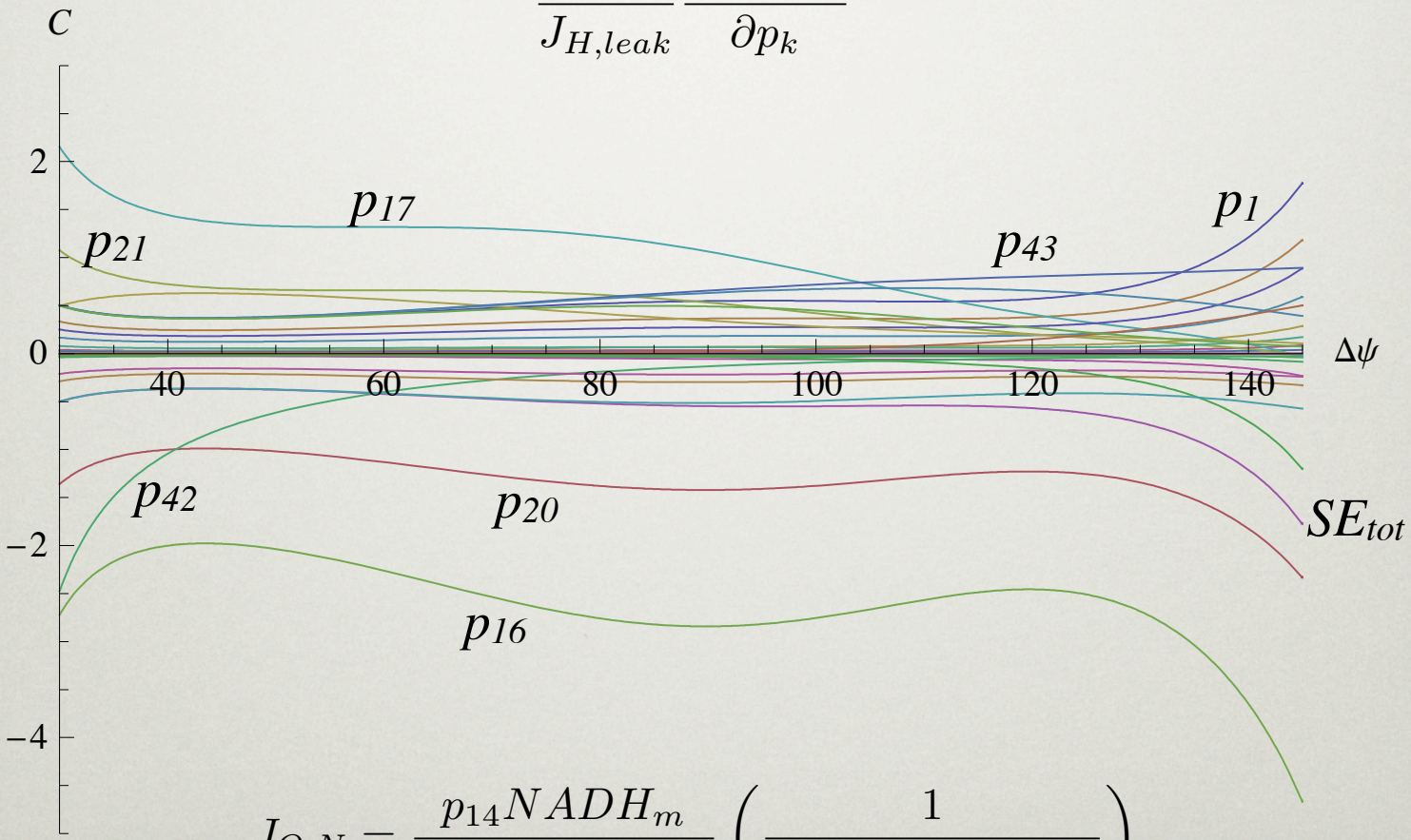
$$J_{H,leak} = p_{41}\left(\Delta\Psi + p_{42}\right) + p_{43}UCP_a$$



Data 1&2: Affourtit, C and MD Brand. *Biochem. J.* **393**:151-159, 2006.
Data 3&4: Echtay, KS *et al*. *Nature*, **415**:96-99, 2002.

Control Coefficients

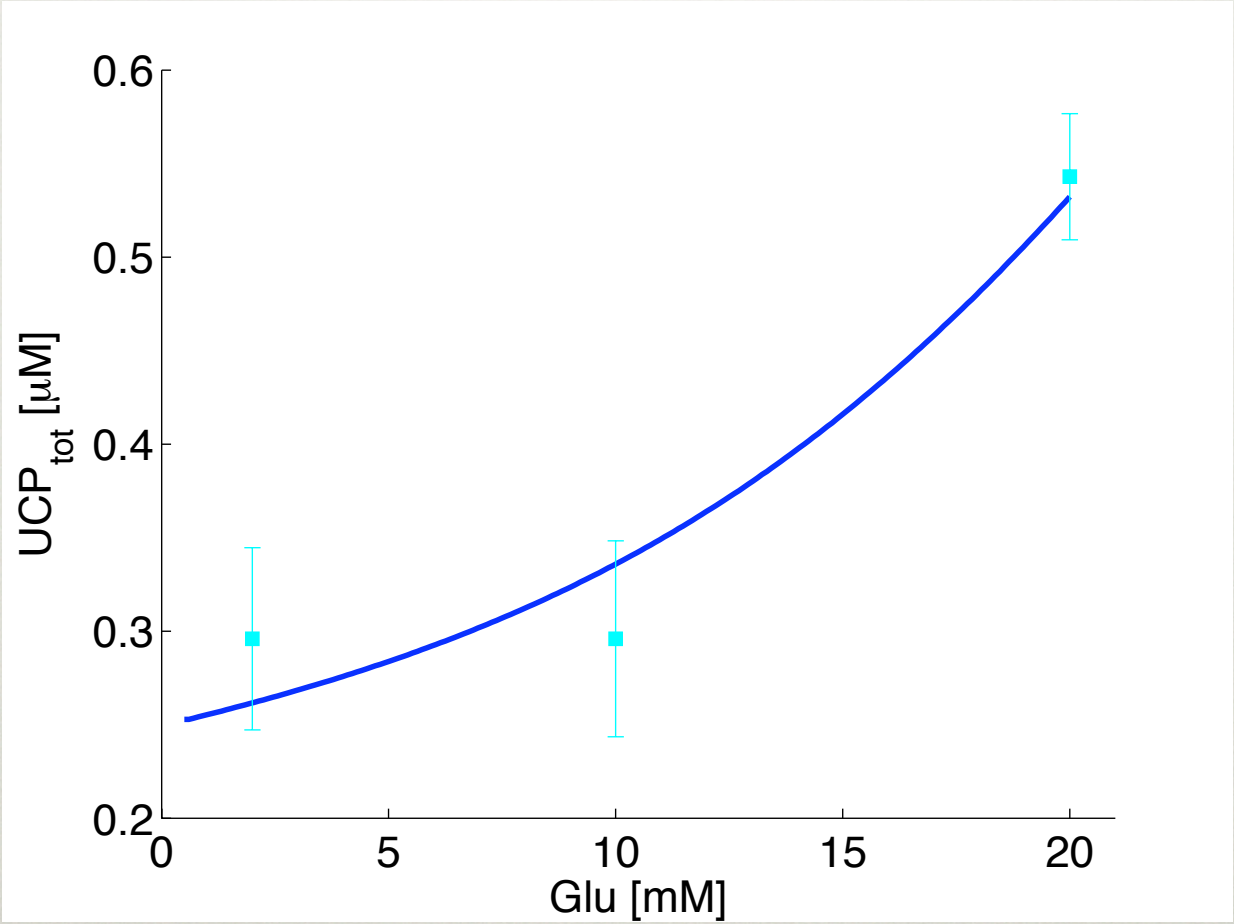
$$\frac{p_k}{J_{H,leak}} \frac{\partial J_{H,leak}}{\partial p_k}$$



$$J_{O,N} = \frac{p_{14} NADH_m}{p_{15} + NADH_m} \left(\frac{1}{1 + e^{(\Delta\Psi - p_{16})/p_{17}}} \right)$$

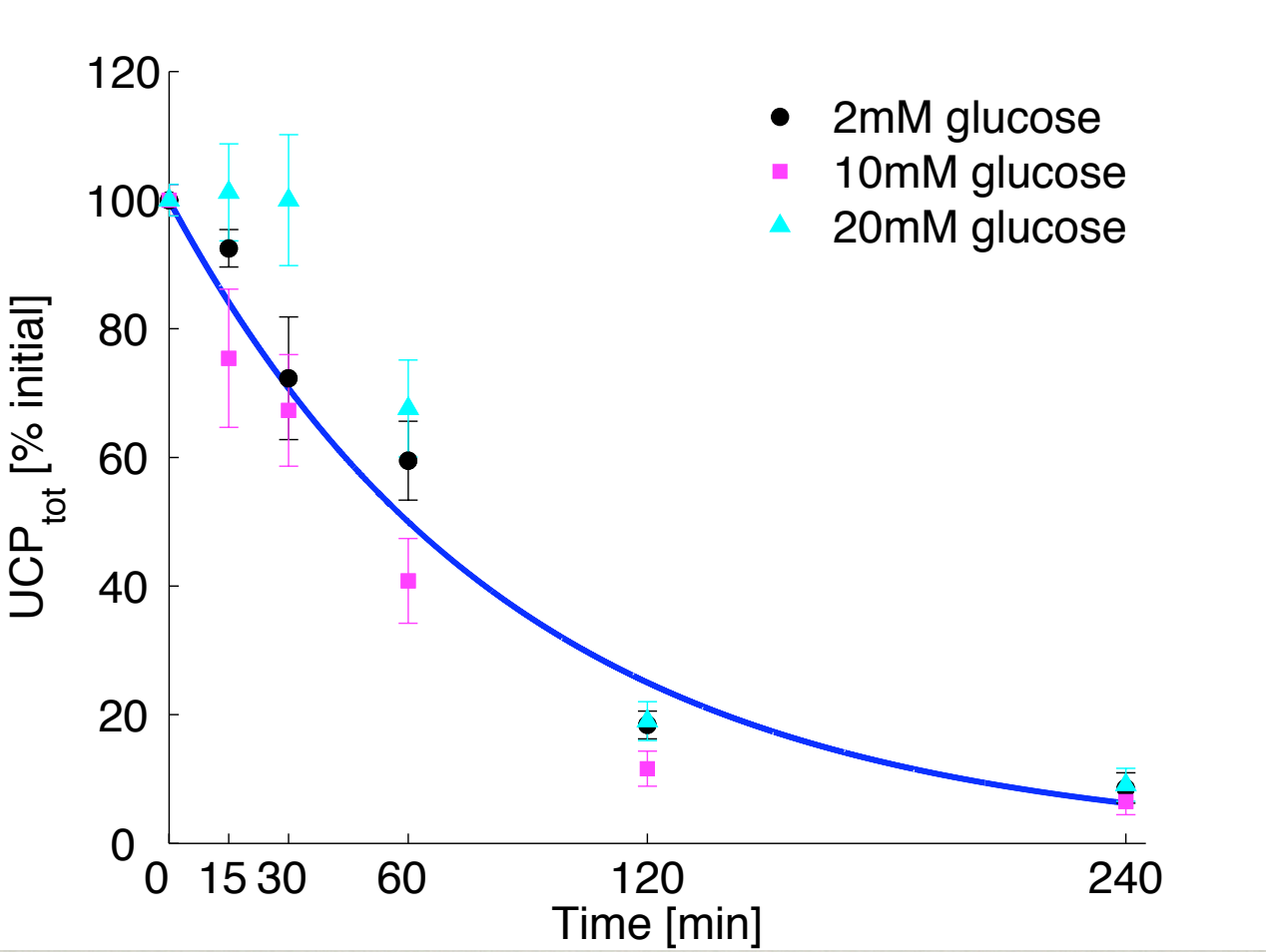
$$J_{H,leak} = p_{41} \left(\Delta\Psi + p_{42} \right) + p_{43} UCP_a$$

UCP concentrations



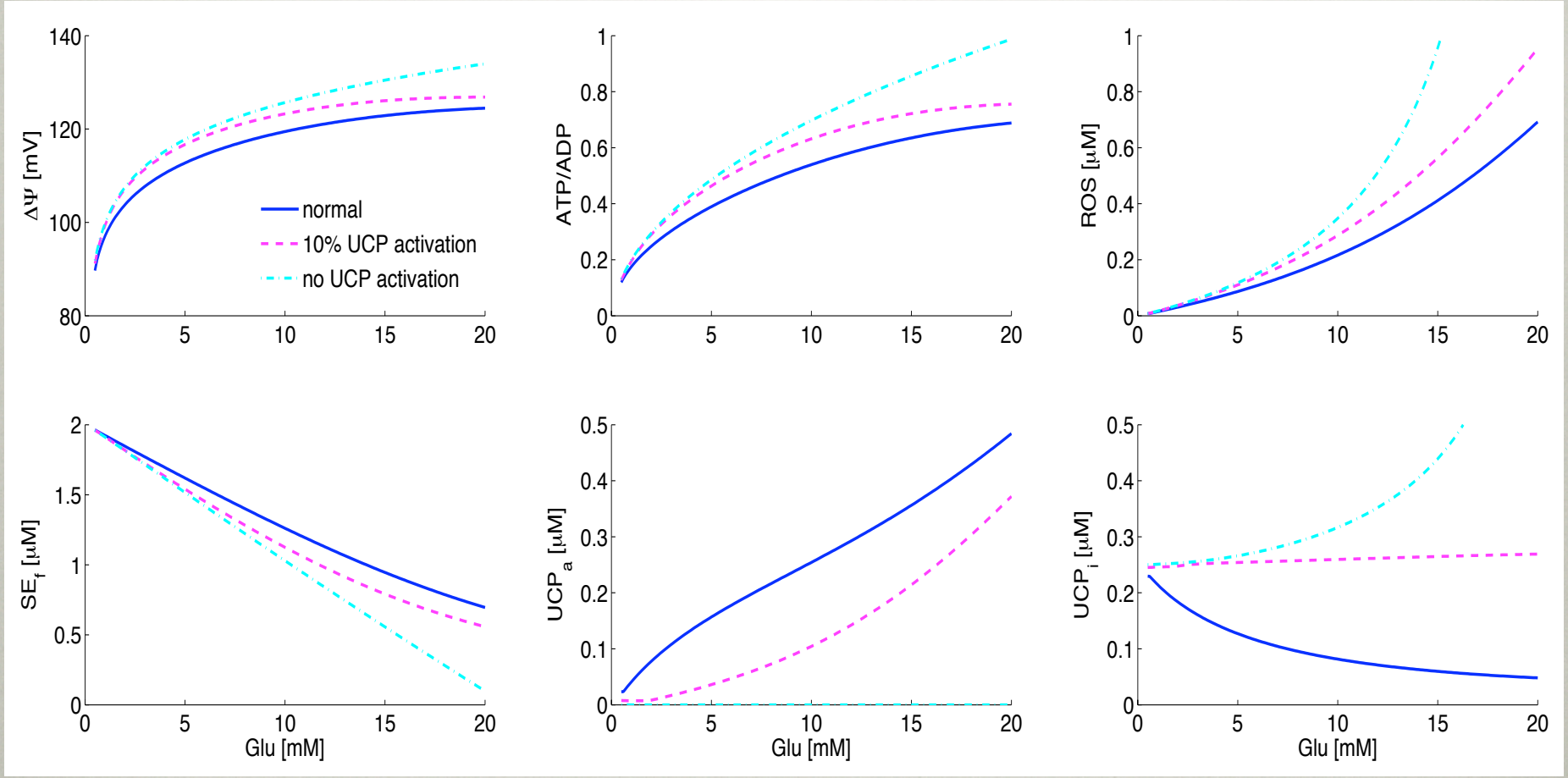
Data from Azzu, V *et al*. *Biochim. Biophys. Acta,* **1777**:1378-1383, 2008.

UCP decay and inactivation

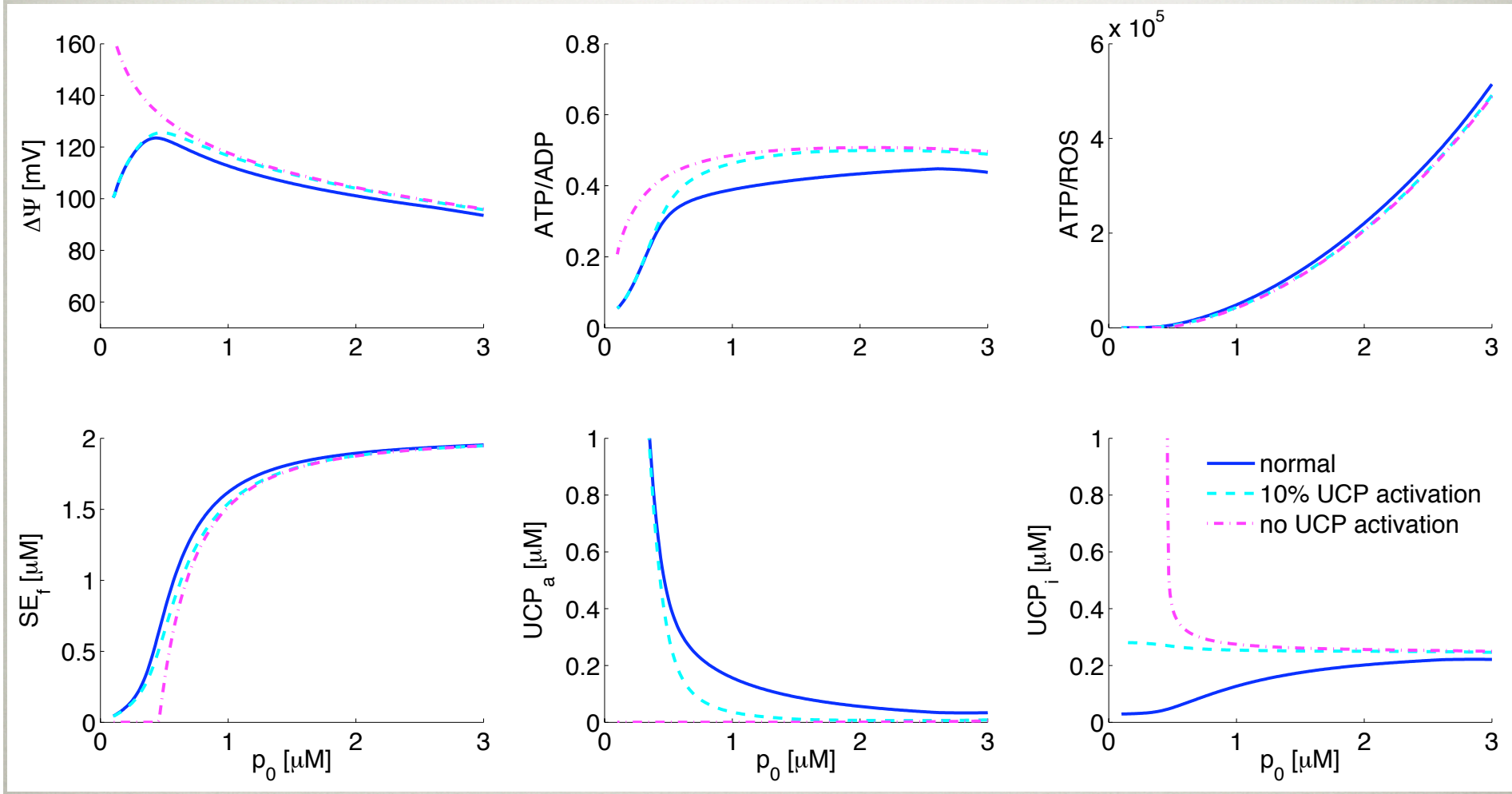


Data from Azzu, V *et al*. *Biochim. Biophys. Acta*, **1777**:1378-1383, 2008.

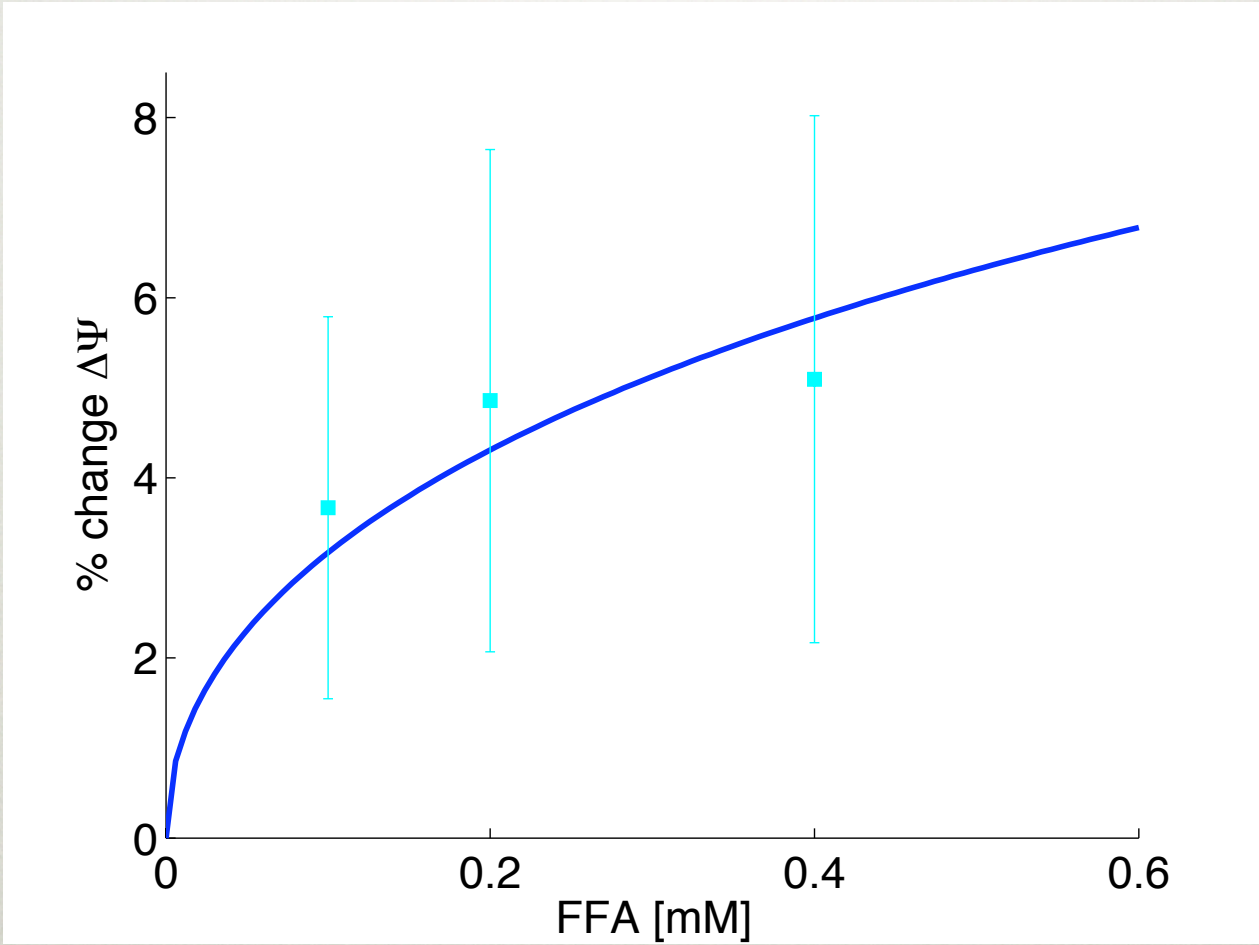
Glucose Dependence and UCP Activation Inhibition



Altering Mitochondrial Density



Fatty Acid Input



Data from Carlsson, C *et al*. *Endocrinology,* **140**:3422-3428, 1999.

Uncoupling Protein 2: A Possible Link Between Fatty Acid Excess and Impaired Glucose-Induced Insulin Secretion?

Nathalie Lameloise,[1] Patrick Muzzin,[1] Marc Prentki,[2,3] and Françoise Assimacopoulos-Jeannet[1]

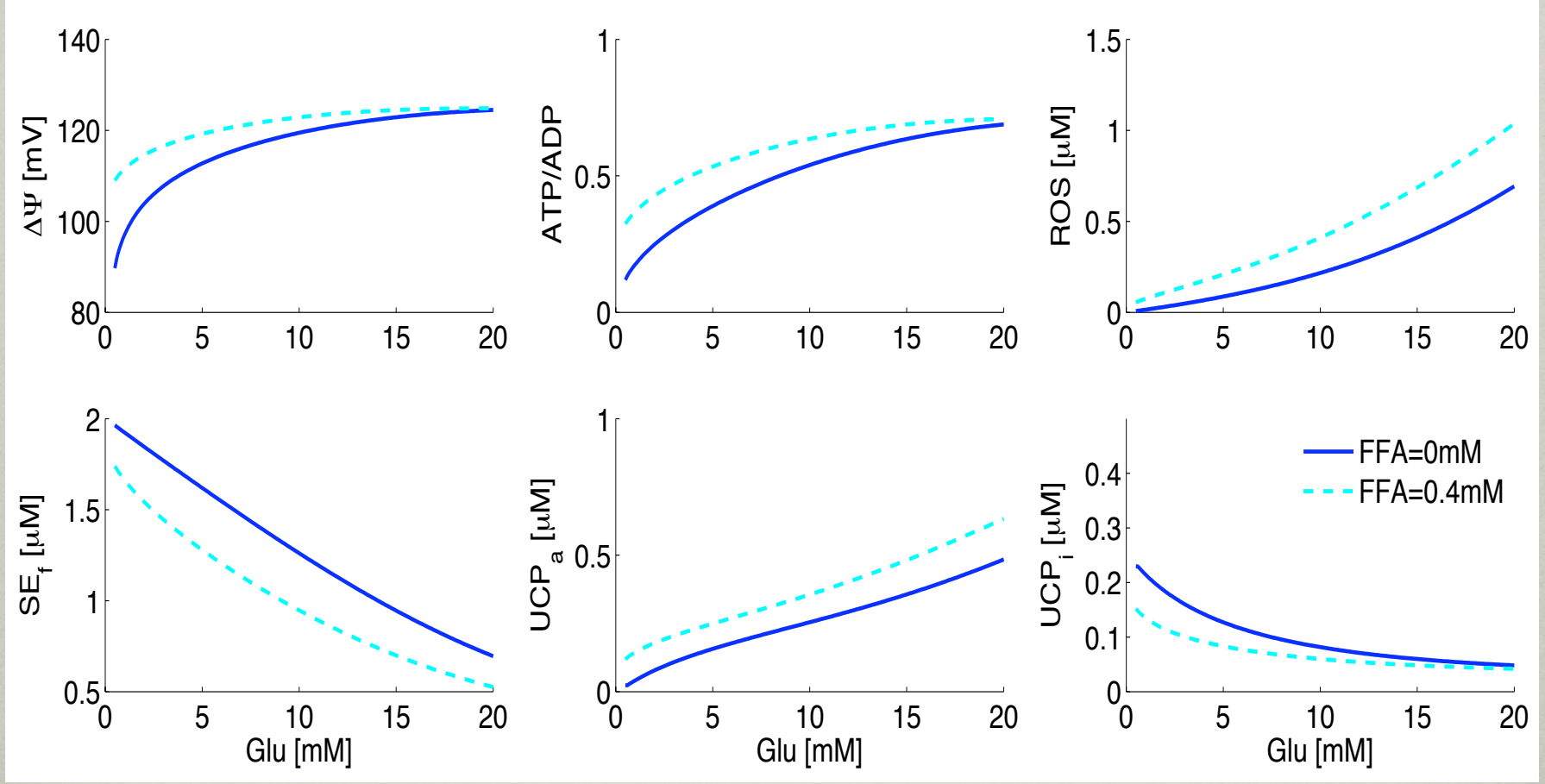
The mechanism by which long-term exposure of the β-cell to elevated concentrations of fatty acid alters

Exposure of INS-1 β-cells to 0.4 mmol/l oleate for 72 h increased basal insulin secretion and decreased insulin release in response to high glucose, but not in response to agents acting at the level of the K_{ATP} channel (tolbutamide) or beyond (elevated KCl). This also suppressed the glucose-induced increase in the cellular ATP-to-ADP ratio. The depolarization of the plasma membrane pro-

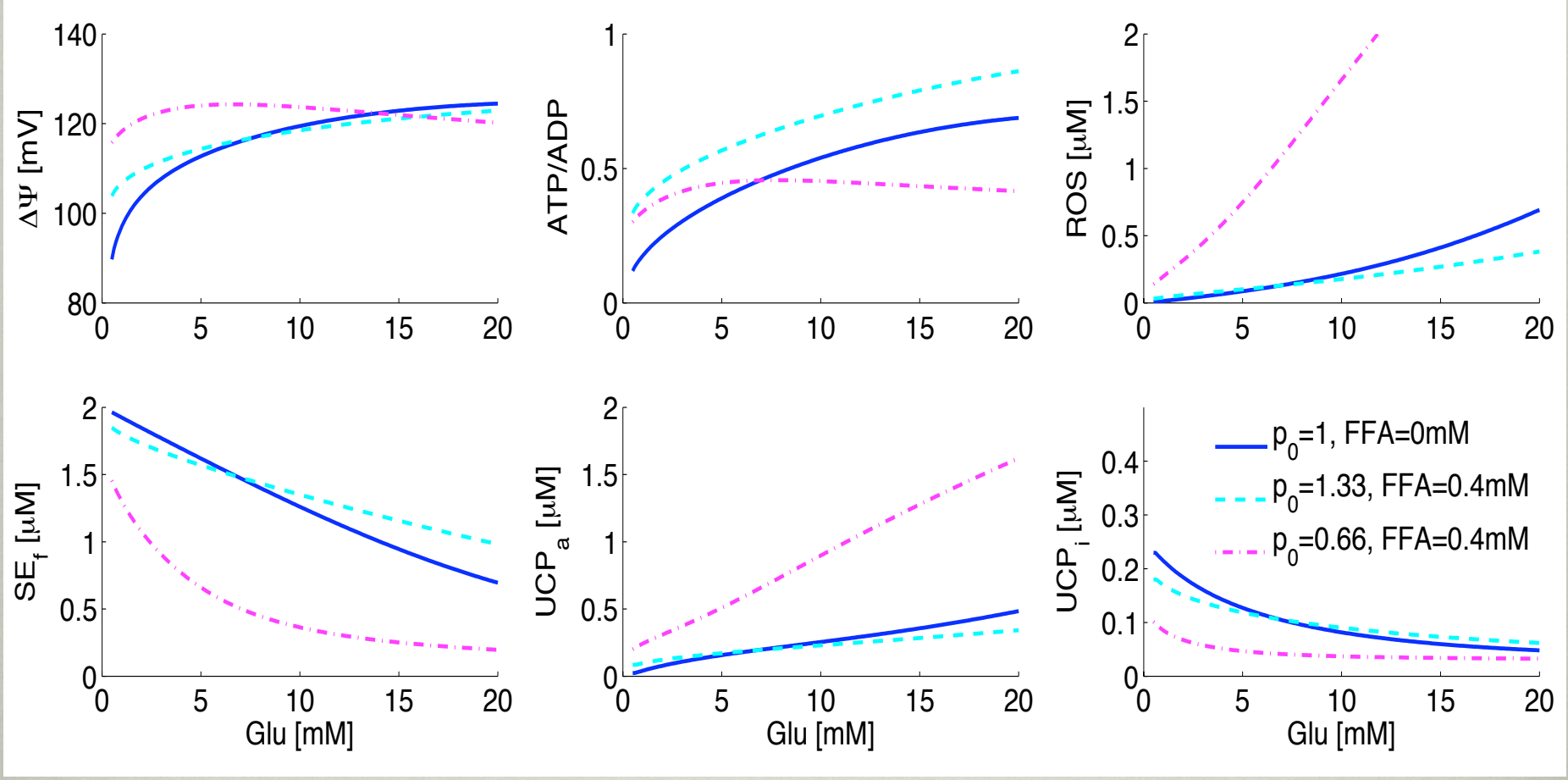
drial membrane potential and a decreased glucose-induced hyperpolarization. The possible implication of uncoupling protein (UCP)-2 in the altered secretory response was examined by measuring UCP2 gene expression after chronic exposure of the cells to fatty acids. UCP2 mRNA and protein were increased twofold

not require their metabolism. The data are compatible with a role of UCP2 and partial mitochondrial uncoupling in the decreased secretory response to glucose observed after chronic exposure of the β-cell to elevated fatty acids, and suggest that the expression and/or activity of the protein may modulate insulin secretion in response to glucose. *Diabetes* 50:803–809, 2001

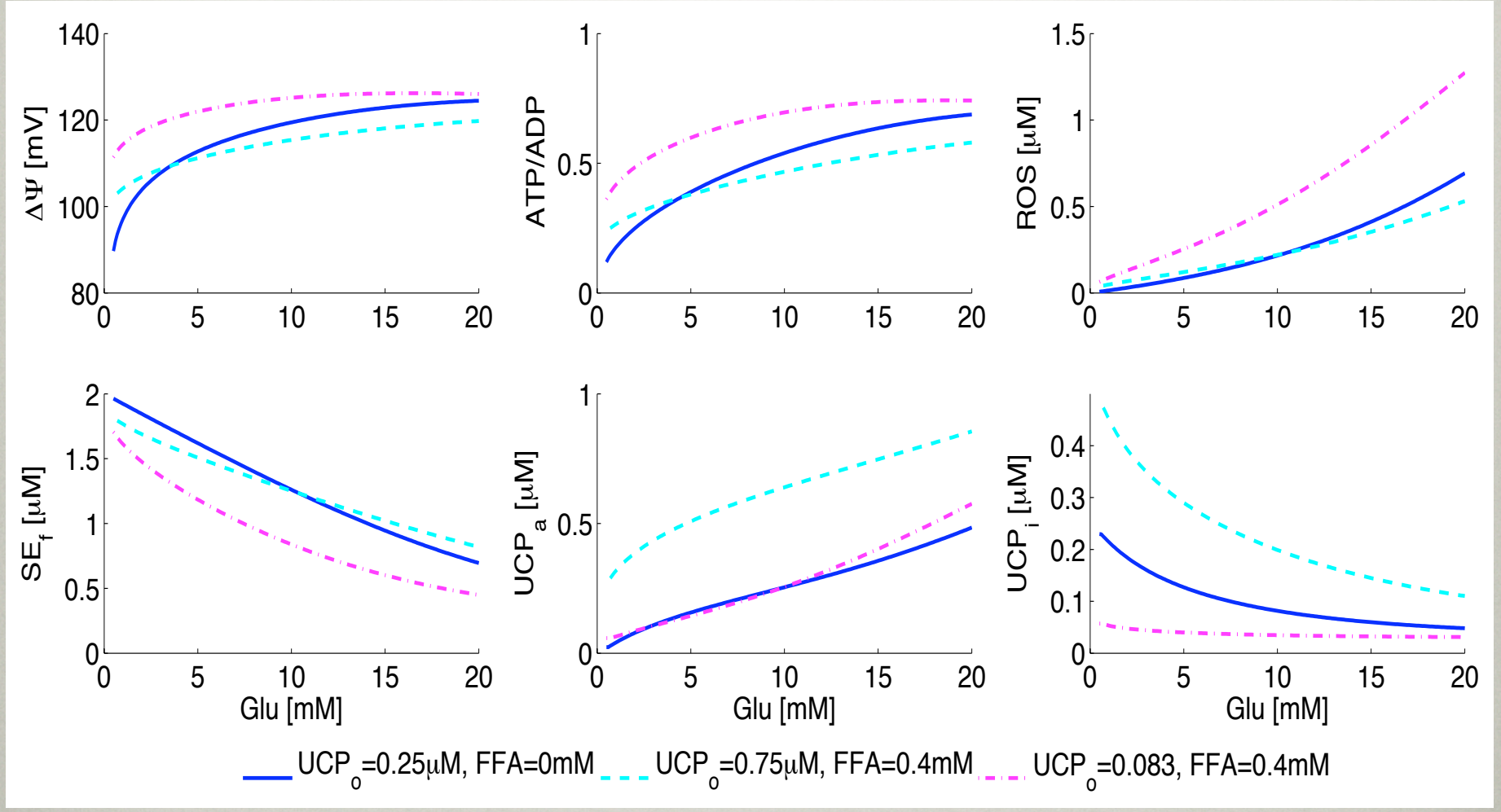
FFA Effect


ΔΨ [mV]
ATP/ADP
ROS [μM]
SE_f [μM]
UCP_a [μM]
UCP_i [μM]
Glu [mM]
Glu [mM]
Glu [mM]
FFA=0mM
FFA=0.4mM

Mitochondrial Biogenesis?

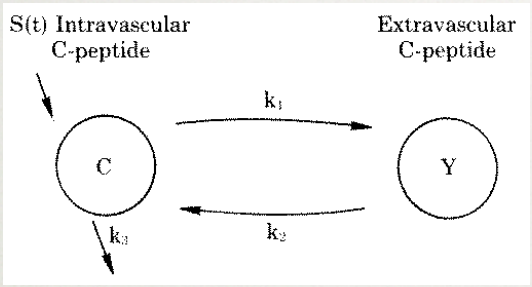


UCP_o?


ΔΨ [mV]
ATP/ADP
ROS [μM]
SE_f [μM]
UCP_a [μM]
UCP_i [μM]
Glu [mM]
Glu [mM]
Glu [mM]
UCP_o=0.25μM, FFA=0mM
UCP_o=0.75μM, FFA=0.4mM
UCP_o=0.083, FFA=0.4mM

A Clinical Application

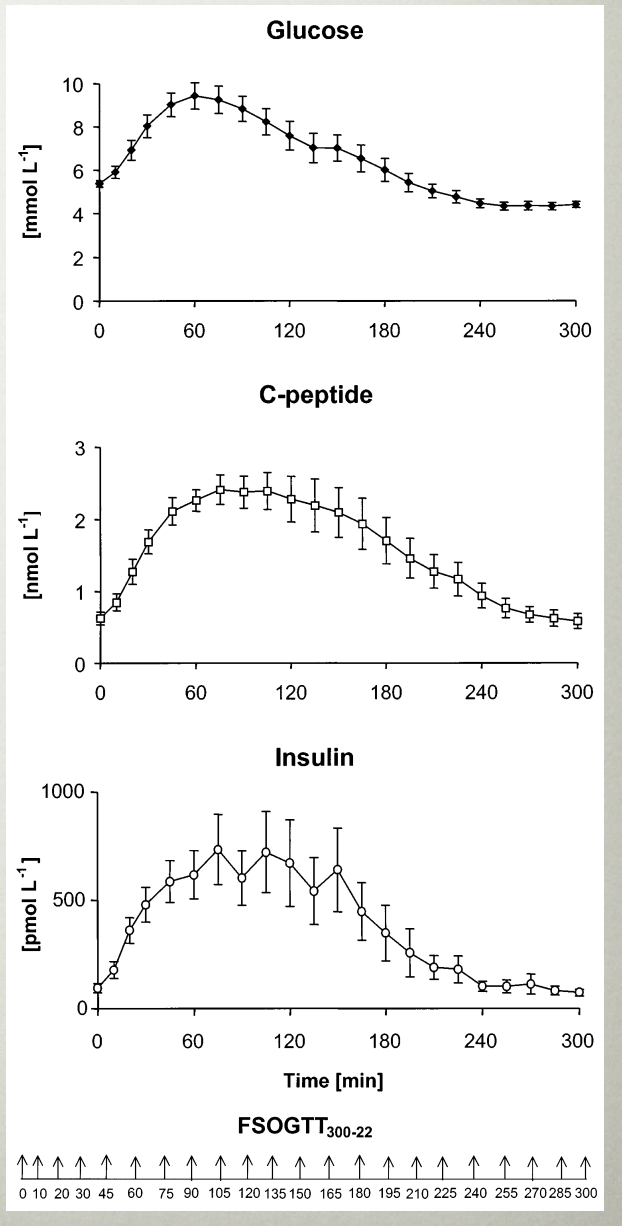
- C-peptide is secreted with insulin.
- The liver extracts insulin from the portal vein.
- C-peptide is not removed by the liver.



$$\frac{dC(t)}{dt} = -(k_1 + k_3)C(t) + k_2 Y(t) + S(t)$$

$$\frac{dY(t)}{dt} = k_1 C(t) - k_2 Y(t)$$

$$S(t) = -k_1 C(t_0) e^{-k_2(t-t_0)} - k_1 k_2 \int_{t_0}^{t} C(s) e^{-k_2(t-s)} ds + \frac{dC}{dt} + (k_1 + k_3)C(t)$$



References:

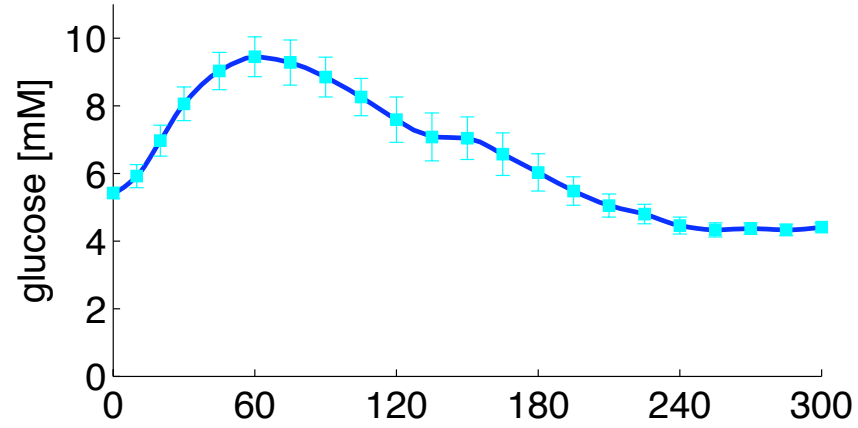
Eaton, RP *et al. J. Clin. Endocrin. Metab.* **51**:520-528, 1980.

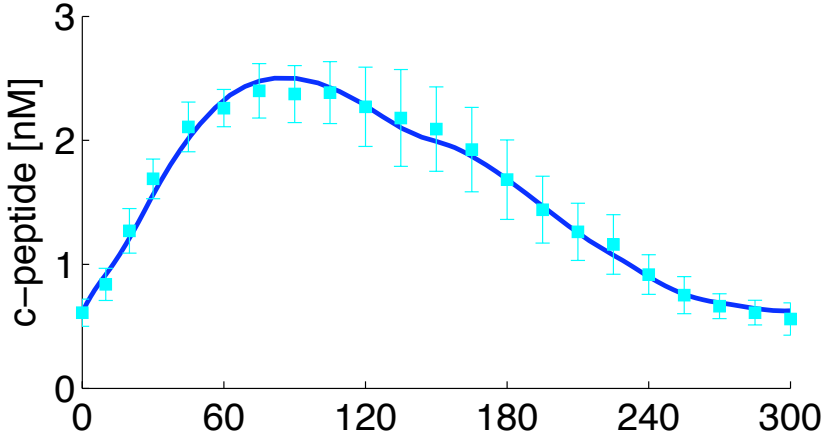
Van Cauter, E *et al. Diabetes*, **41**:368-377, 1992.

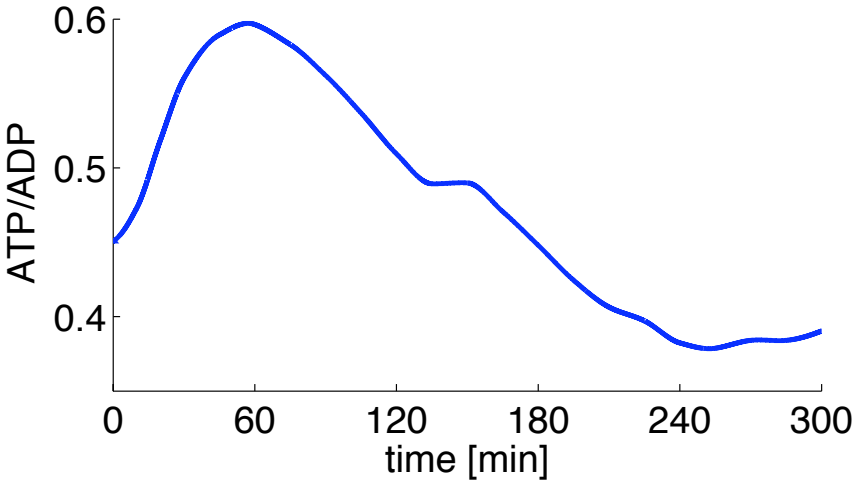
Breda, E *et al. Diabetes,* **50**:150-158, 2001.

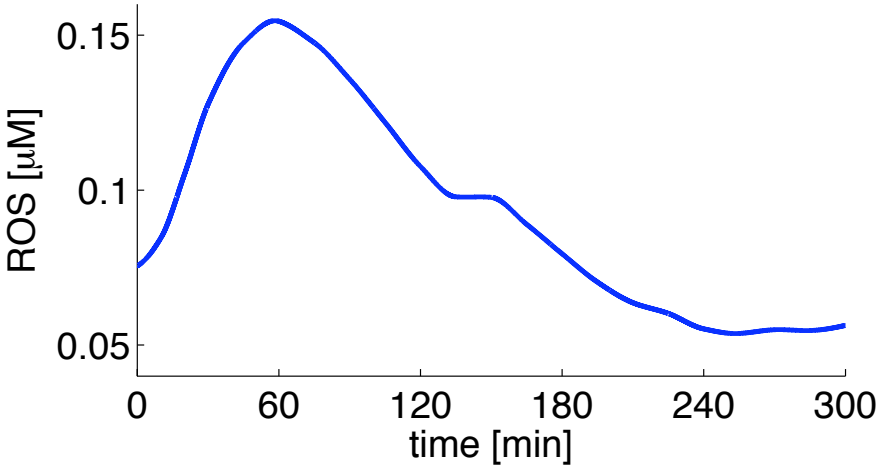
Insulin Secretion Model

$$S(t) = k_a \frac{ATP}{ADP} + k_r ROS - k_b$$









Data from Breda, E *et al*. *Diabetes*, **50**:150-158, 2001.

Conclusions

- The model we developed is capable of predicting mitochondrial responses to nutrient inputs (glucose and fatty acids).
- In the pancreatic β-cell, mitochondria (ROS, UCP, antioxidants) play a central role in cellular function (or dysfunction) as it relates to insulin secretion.
- The model can be generalized to mitochondria in other tissues.
- Mitochondria serve a systemic function: one that can suggest holistic metabolic therapies.

Acknowledgments

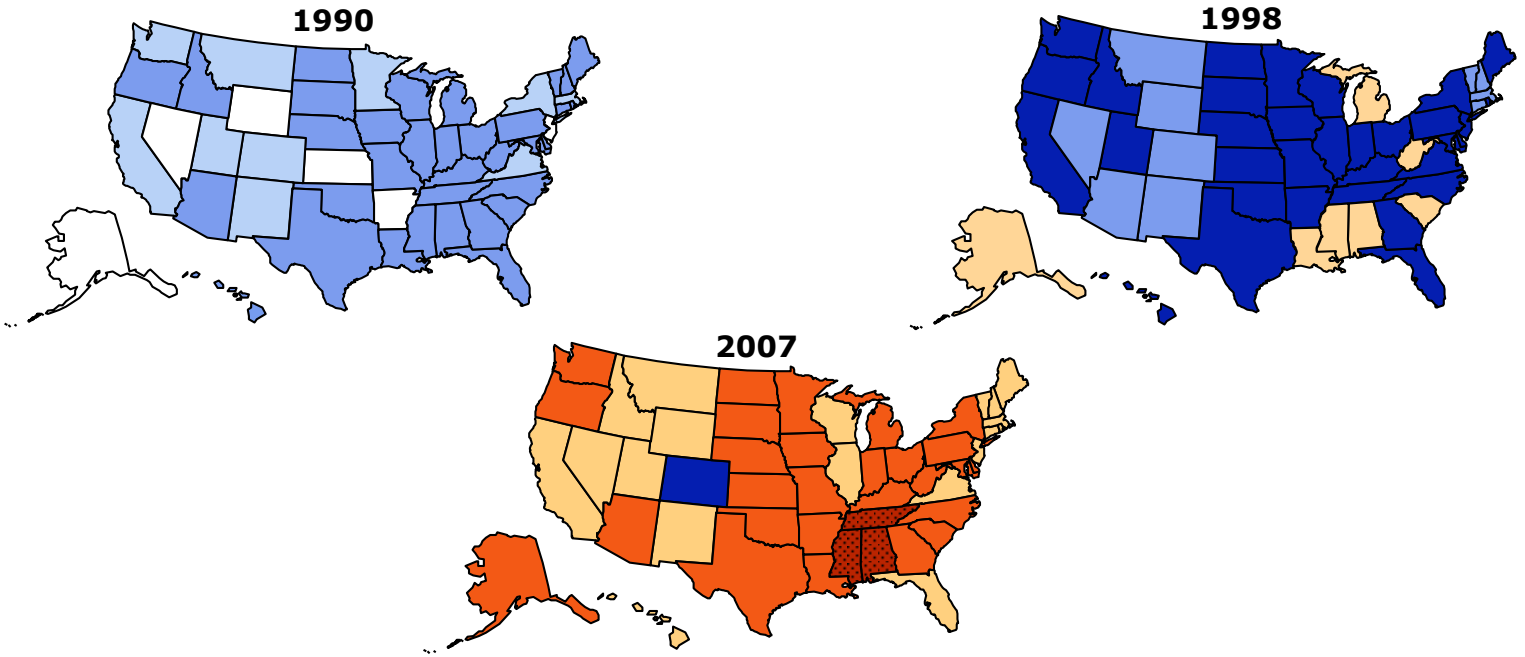
Supervisor: Vipul Periwal

Work supported by the Intramural Research Program, NIDDK, NIH.

Obesity Trends* Among U.S. Adults

BRFSS, 1990, 1998, 2007

(*BMI ≥30, or about 30 lbs. overweight for 5'4" person)



Source: CDC Behavioral Risk Factor Surveillance System.